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ANTIHISTAMINE AGENTS IN ALLERGY

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ANTIHISTAMINE AGENTS IN ALLERGY*

Conference Chairman
Fredrick F. Yonkman

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INTRODUCTORY REMARKS

By Fredrick F. Yonkman

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On behalf of the Organizing Committee, cooperating with Mrs. Eunice Thomas Miner, Executive Director of The New York Academy of Sciences, I should like to express my pleasure in introducing this monograph. Thanks are due to Dr. Dragstedt and the late Dr. SubbaRow for their generous and able assistance in the construction of the conference and especially to Mrs. Miner for attending so efficiently to the many details that are part and parcel of a conference of this scope.

As a Committee, we are most appreciative of the excellent suggestions regarding participants in the conference, both as to speakers and discussants, and we extend our genuine gratitude to the several anonymous supporters who made possible the assembling of our illustrious speakers, whose workshops and clinics are so widely separated geographically. Our chief regret is that numerous other renowned contributors to the scientific advances in this particular field could not have been invited to participate. There were reasonable limits which we could not exceed, especially those of time and support. Had both been adequate, however, certainly it would not have been difficult to have extended the conference for a longer period.

As one scans the Table of Contents of this monograph, one appreciates the historical developments concerning histamine, its pharmacodynamics, and its varying role in anaphylactic and allergic situations. We are aware of the devious methods whereby man has sought to dampen histamine's physiologic behavior. In one phase, it was degraded or split into its components; in another, it was coupled with cooperative partners. More recently, however, it is being given the "physiological brush-off" by handy whiskbrooms such as are now known, correctly or incorrectly, as "antihistaminic agents." What is next? If histamine is a chief offender in anaphylaxis or allergy, will someone some future day manage to dampen its activities by means other than those now at our disposal? This is highly probable, and one is almost sufficiently foolhardy to venture the prediction that such will be the case. What the next approach will be is problematic, but perhaps some clues may be found among the papers included in this monograph.

Recently, there has been some criticism of the term "antihistaminic" as employed for the various derivatives of the original Fourneau compounds. Much of this criticism seems warranted, since there is ample evidence that many of these substances exhibit other prominent pharmacodynamic actions. They are, to a varying degree, anticholinergics, local anesthetics, central sedatives, or oddly enough, in some instances, analeptics and even potentiators of certain physiologic responses. In fact, these new agents can manifest many actions simultaneously. Justly one asks why they are classified as antihistaminics. With equal justification, one might inquire as to how they might otherwise be classified. Since their chief pharmacodynamic characteristic is that of nullifying the action of histamine, they are logically identified as antihistaminic agents, whereas, if one chooses to categorize them from a therapeutic point of view, they probably should be classified as antiallergic agents along with epinephrine, ephedrine, and similar compounds.

Although most of the more recently developed synthetic variations of the original Fourneau and other compounds exhibit varying but marked capacities for nullifying the physiologic effects of histamine, their somewhat similar potency in terms of antianaphylactic action is not only fascinating but challenging. This variance in antihistaminic but not in antianaphylactic potency is, however, no more provocative of thought than is the finding of Abramson and coworkers in their experiments dealing with iontophoretic administration of histamine to the skin. By *reversed* iontophoresis, the histamine was detectable in the dermal fluid thus obtained. When ragweed pollen extract was administered in the same way, however, allergic whealing resulted, but then, after *reversed* iontophoresis, the dermal fluid contained no histamine or H-substances. Failure to detect histamine or H-substances in the fluid could reflect a true absence of these compounds, but it remains to be proved that they were not present because of possible linkage in a conjugated or nonreactive form, a condition which could militate against detection. Granted, however, that free histamine or active H-substances were not present in such dermal fluid, such an absence of suspected offenders challenges the concept that they are entirely or chiefly responsible for the whealing reaction or, for that matter, responsible for any of the so-called "allergic" manifestations. One is still more puzzled by the fact that such whealing reactions can be alleviated, prevented, or diminished by the new antiallergic drugs known as "antihistaminic agents." What may be the mechanisms involved? We trust that some light has been shed upon this problem by the papers included in this monograph. But, in the event we find ourselves asking many more questions, most of which will remain unanswered, we may find comfort in the words of Robert Louis Stevenson, "To travel hopefully is a better thing than to arrive, and the true success is to labor."

THE PHARMACOLOGY OF HISTAMINE: WITH A BRIEF SURVEY OF EVIDENCE FOR ITS OCCURRENCE, LIBERATION, AND PARTICIPATION IN NATURAL REACTIONS

By Sir Henry Dale

Chairman, Board of Trustees, Wellcome Physiological Research Laboratories, Kensington, London, England

It was in August, 1907, while watching a demonstration at an International Physiological Congress at Heidelberg, that I first became conscious of the existence, in certain extracts of ergot, of a potent substance different from any of those which we had hitherto encountered in that drug. Kehrer (1908), an obstetrician of Heidelberg, had been working in the department of Professor Magnus there and had found that Magnus's method of studying the reactions of surviving loops of intestine, suspended in a saline solution, was applicable to the horn of a cat's uterus. He had tested a number of substances for their action in this, including, as was most natural, a series of preparations of ergot. Of these he had found one to be pre-eminent in its prompt and powerful stimulant effect, of which he gave a striking demonstration. This was the so-called *Ergotinum dialysatum* of Wernich. Knowing something already, by that time, of the occurrence in ergot extracts of bases recognized elsewhere as products of bacterial decomposition, and finding, on inquiry, that Wernich's method of preparing his extract by dialysis was such as to provide optimal conditions for an active putrefaction, I was not inclined to accept Kehrer's conclusion that his test demonstrated the real active principle of ergot, or that it selected the extract of that drug which would be the most valuable in the clinic, with ordinary methods of administration. Indeed, it was not until 28 years later that the specific alkaloid of ergot of most value in practical obstetrics was at last discovered.

Meanwhile, in 1907, it was clear that Kehrer's test had revealed the presence, in a particular ergot extract, of something with an intense and immediate stimulant action on the tone of isolated uterine plain muscle, and different from any of the substances which had then been found in ergot. It seemed likely, on previous experience, to be an amine produced by putrefaction from some amino acid. This news I carried back from Heidelberg to London and to my late friend, George Barger. We had a batch of the *Ergotinum dialysatum* prepared for us. Our colleagues at the factory confirmed, with some emphasis, the suspicion that the process would be attended with extensive putrefaction. From it, with the guidance of my physiological tests, Barger isolated histamine (Barger and Dale, 1910). Just as we had obtained it, and before we had quite completed its identification, Ackermann (1910), of Würzburg, published an account of his preparation of histamine from histidine by deliberate putrefaction. When, eventually, we obtained a specimen from him, it enabled us to complete the identification of the base which we had isolated from the ergot extract. Then we learned that Kutscher, of Marburg, had been making the same isolation independently, at the same time; though a few comparative physiological tests, which Kutscher (1910) made of his ergot base, led him to doubt its complete identity with histamine. The source of this apparent discrepancy was not difficult to discover, however, and there was soon no doubt about the identification.

In any case, histamine was not a new substance. Windaus and Vogt (1907) had already prepared it by synthesis in 1907, though they had no suspicion of its physiological activity or of its potential interest. Even the precise identity of the base from ergot was a point of no more than passing concern. The matters of real interest, and those which, by a natural development, have led to this monograph, were: the nature of the action of histamine itself as it came into view, when my late friend, Laidlaw, and I (Dale and Laidlaw, 1910, 1911) began to work on it in detail; later, its demonstrable presence in the body, apparently as a natural constituent of most living cells, but in widely different proportions in those of different tissues; and, lastly, the conditions of its release from these, in a form enabling it to produce its intense and characteristic actions on such cells as are sensitive to its effects, particularly on the cells of plain muscle and endothelia. All these belong to the pharmacological, physiological, and pathological background of this monograph on the "antihistamines." What follows is, at the sacrifice of much detail and without any attempt at completeness in crediting particular items of knowledge to their discoverers, a general survey of this background.

Actions and Occurrence of Histamine

If we had dealt with its actions only as these were displayed on isolated organs, we should have described histamine simply as a powerful stimulant of involuntary muscle. Even isolated strips of any arteries which were large enough to furnish such preparations, from any of the species from which we tried them, contracted vigorously when histamine was applied in high dilutions. Yet, if histamine was injected, in doses between one half and one thousandth of a milligram, into the vein of an anesthetized cat or dog, it caused an immediate steep fall of the systemic arterial pressure, which could easily be shown by plethysmographic methods or by recording venous outflow to be due to simple vasodilatation in the limbs and in most abdominal viscera alike. The kidney alone seemed to provide an exception, since it shrank with the histamine depression more than could be accounted for by a mere passive accommodation of its volume to the general fall of arterial pressure. If, however, we took from a cat an organ which characteristically showed a vasodilatation in response to histamine thus injected into the natural circulation, whether a limb or a loop of intestine, and perfused it artificially with a saline solution or with blood, we were, for a long time, unable to demonstrate any effect of histamine but simple vasoconstriction, when it was injected into the perfusion stream in any dose which was effective at all. We had to leave that paradox unresolved for the time being, though the explanation of it, when it came some years later, provided a clue to the nature of some of the most important actions of histamine.

The actions of histamine injected into the general circulation, and especially the contrast between the syndromes which it thus produced in different species, showed, further, a most interesting and suggestive resemblance to the similarly contrasted syndromes of the anaphylactic shock, as these had then recently been described in the different species in which this reaction had been specially studied. In the anaphylactic shock as seen in the dog, a phenomenon which had first been encountered and described by Portier and Richet (1902), the central feature was a venous turgescence of the liver, with congestion of the abdominal alimentary canal and a profound collapse of the systemic arterial pressure. In a guinea pig, on the other hand, into which the second shock-releasing dose had been injected intravenously without anesthesia, though effects on the liver were not excluded, the picture of the shock was dominated by a valve-like, asphyxiating constriction of the bronchioles, leading to rapid death with acute pulmonary distention (Auer and Lewis, 1910).

Biedl and Kraus (1910) had drawn attention to the very remarkable correspondence between the effects of the well-known Witte's peptone—the product of an artificial peptic digestion of fibrin—when administered intravenously under similar conditions to the normal dog and guinea pig, and those produced in anaphylactic animals of the same two species by the injection of the sensitizing antigen. With either sensitizing antigen or peptone, the dog showed the acute hepato-enteric congestion and the profound collapse of the systemic arterial pressure, while either reagent in the guinea pig produced acute spasm of the bronchioles and death from the acute, asphyxiating distention of the lungs.

The immediate tendency had been, not unnaturally, to attribute the anaphylactic symptoms to the initiation, by the union of antigen and antibody in the blood, of a rapid proteolysis, presumably of the foreign antigen, liberating an unspecific, poisonous cleavage product acting like the familiar Witte's peptone. In the ensuing years, a relatively enormous literature accumulated, presenting a mass of detailed evidence which could be plausibly interpreted in terms of this conception of the proteolytic liberation of an "anaphylatoxin," a hypothetical "split product," formed, according to the particular variant of the theory adopted, by cleavage either of the antigen or of the proteins of the victim's own blood plasma and somehow initiated by the union of antigen and antibody. In the general examination which Laidlaw and I made of the action of histamine on these same animal species, it was impossible to miss the resemblance between the contrasted effects which this substance also produced in the dog and in the guinea pig and those produced either by Witte's peptone or by the anaphylactic reaction in the same species. Histamine again produced a profound collapse of the systemic arterial pressure in the anesthetized dog and a valve-like constriction of the bronchioles and consequent asphyxiating distention of the lungs in the unanesthetized guinea pig as the predominating features of its actions in the respective species under these conditions. In the rabbit, the correspondence seemed, for a time, to break down. Laidlaw and I had found that histamine killed the rabbit by causing an obstructive constriction of the branches of the pulmonary artery, with consequent distention and failure of the right side of the heart; whereas death of the rabbit in the anaphylactic shock had been attributed to a directly poisonous action on its heart, which we had found to be only mildly stimulated by histamine when perfused as an isolated preparation. Some years later, even this apparent exception disappeared, when Dr. Coca (1914) reinvestigated the cause of anaphylactic death in the rabbit and found it to be due, in fact, to intense pulmonary arterial constriction, causing the right side of the heart to fail from overdistention, just as Laidlaw and I had found with histamine.

The different items of evidence, as thus stated, could clearly be reconciled by identifying the anaphylatoxin with histamine, and there were not wanting those who were ready to put forward so obvious a suggestion. I am glad, on the whole, that Laidlaw and I refrained from such easy speculation, and that we were concerned rather to note the absence from the histamine syndrome of some of the characteristic features of the anaphylactic and peptone shocks. Histamine caused but a relatively mild swelling of the dog's liver, with no conspicuous congestion of the bowels and no loss of the coagulability of the blood, the latter occurring in both dog and guinea pig as a feature of the anaphylactic reaction. Even if there was indeed an anaphylatoxin—and it should be noted that there never was any really direct evidence for it—histamine could not, therefore, be identified with it. It could, at most, be only one constituent of a complex reagent, the other effects of which must be due to other constituents. We ourselves could get no further than the conclusion that the production by histamine of so large a part of the effects constituting the anaphylactic and peptone shocks must have *some* meaning, but that there were no sufficient data, at that time, even for speculation as to what that meaning might be.

Another correspondence to which we drew attention was that between the actions of histamine and those of "depressor principles," which various authors, from Vincent and Sheen (1903) onwards, had described as existing in extracts from many organs of the body and had distinguished from choline by the resistance of their effects to atropine. Popielski (1909) had postulated the existence of a widely distributed "vasodilatin" of this kind, found in organ extracts and, as he believed, also in Witte's peptone. Barger and I (1911) actually isolated histamine from an extract of fresh intestinal mucosa, in sufficient quantity to account for its atropine-resistant depressor action. The choice was, perhaps, an unfortunate one, since it was impossible to exclude completely the suspicion that histamine from such a source might be a product of bacterial action.

Let me try now to trace separately, in briefest outline, the further developments which have resulted from following the different clues presented by these preliminary studies.

Effects of Histamine on Vascular Tone. Take first the paradox of histamine acting as a systemic vasodilator in the cat and dog when injected into the normal circulation and as a simple vasoconstrictor on isolated arteries or artificially perfused organs from the same species. In later investigations (Dale and Richards, 1918, 1927; Burn and Dale, 1926), this discrepancy was traced to the maintenance, under the conditions of normal circulation, of a tone of the minute blood vessels, the ultimate arterioles, and capillaries, which histamine relaxes; whereas, on somewhat larger arterial branches, its action is one of pure vasoconstriction. This tone of the minute vessels is not upheld by nervous action alone, since it is present, and is apt even to be maintained with conspicuous efficiency, in an organ deprived of its nerves by degeneration. To what constituent of the normally circulating blood it is then due is a point which is still unsettled, and one which might well be worthy of further investigation.

In our more recent experiments, with no clearly identifiable change of technique to account for it, we have found that, early even in a perfusion, with plain blood either defibrinated or kept from clotting with heparin, a tone is often built up spontaneously in the vessels of the perfused organ which, from other experiments, we can identify as a tone of the smallest vessels and which histamine then relaxes as in the normal circulation. Later in such an experiment this spontaneous tone disappears, and the vasodilator action of histamine is then replaced by the constrictor effect, which was, at one time, the only one we were able to observe with artificial perfusion. More recently, again, in unpublished experiments made during the war, I have consistently observed the appearance of this tone of the minute vessels, which histamine relaxes, in cats' legs perfused with saline solutions of hemoglobin.

In the perfused organ, this early, spontaneous tone of the minute vessels later disappears, so that histamine then causes pure vasoconstriction. A tone can then be regenerated and the dilator effect of histamine recovered by adding adrenaline (Dale and Richards, 1918) or post-pituitary vasopressin (Burn and Dale, 1926) in high dilution to the perfusing blood. Throughout the cycle of changes thus produced, it is to be noted that ordinary arterial vasodilators, like the nitrites or acetylcholine, have consistently produced only vasodilator effects, even when that of histamine has been replaced by simple vasoconstriction.

Under normal conditions, in the arterial arborization, the level at which the reaction to histamine changes from constriction to dilatation varies in the different species. In the dog, it seems to occur relatively early in the branching, so that a preparation of the mesenteric arteries can be obtained from that animal in which the tone of visible arterioles still relaxes in response to histamine. Also, the tone which histamine relaxes in perfused organs from the dog is correspondingly more resistant to the conditions of artificial perfusion. A few experiments (Burn and Dale, 1926) suggest that the level of the change in the monkey is probably also such as to include in the dilator effect of histamine arterioles larger than the most minute. In the cat, the histamine dilatation appears to be limited to the ultimate arterioles and capillaries, and in the rabbit, to be so completely confined to the capillary bed that it was only under special conditions that Feldberg (1927) found it possible to demonstrate its effect in a rabbit anesthetized for recording on the arterial pressure. Under ordinary conditions of anesthesia, as with ether or urethane, the rabbit's systemic arterial pressure responds by a simple rise to any dose of histamine which is effective at all, the effect being that of a simple arterial constriction. Only under light anesthesia with chloralose is this replaced by a moderate and relatively slow depressor effect. Presumably this is due to a relaxation of a capillary tone, constituting the major component of resistance under these conditions. Its nature can be studied directly, without anesthesia, in the completely denervated ear of an albino rabbit held against the light. When a small dose of histamine is injected into a vein of the other ear of such an animal, the constriction of the arteries down to the smallest visible ones and the simultaneous flush of the tissue between them, due to dilatation of the capillaries, can readily be seen. In general terms, then, and with the differences indicated in the different species, we arrived at a conception of the histamine vasodilatation, which had presented such anomalies, as differing from other vasodilator effects only in being limited to the finer twigs of the arterial tree, if it affected the arteries at all, and in extending, in all species, to the capillary network and producing on these at least a large part of its vasodilator effect.

Experiments by Sollmann and Pilcher (1917) showed that histamine, applied to the slightly abraded human skin in dilutions down to 1 part in 100,000, causes local reddening and an urticarial wheal, suggesting that, if the capillary dilator effect had more than a minimal and transient intensity, it caused an abnormal permeability of the capillary walls. This suggestion contributed to the interpretation of the irreversible, shock-like failure of the circulation produced in the anesthetized cat by larger doses of histamine, which I was studying during the first world war with Laidlaw (Dale and Laidlaw, 1919), in continuation of the experiments with A. N. Richards. We found that, in this condition, in spite of strong contraction of the visible arteries, the circulation was failing from a deficient volume of blood in currency—an oligaemia. As much as one half the plasma had leaked through the walls of the permeable capillaries, and a large proportion of the remaining, thickened blood had become stagnant at the periphery in the relaxed capillary bed, so that the still vigorous heart had only a small volume to eject at each beat. Here, again, the total histamine effect could be interpreted as presenting, in a more intense and persistent form, the complex of vasoconstriction of all but the minutest arteries, with a general relaxation of the capillary network, involving a large increase in its effective capacity and an abnormal permeability of its endothelial walls. As might be expected, in the dog, the collapse of arterial pressure caused by histamine involved a larger component of relaxation of arterioles and a smaller component of capillary dilatation, loss of plasma, and oligaemia.

Simultaneously with these observations, Krogh (1929), by a series of elegant, direct observations, was arriving at conclusions which greatly reinforced and supplemented our own, as to the existence of a normal capillary tone keeping a large proportion of the network closed in a resting tissue, its physiological variability, and the increase of the permeability of the endothelial walls of the capillary vessels with relaxation of this tone. Sir Thomas Lewis was beginning, at the same time, his series of masterly studies of the reactions of the small blood vessels of the human skin (later embodied in his classical monograph on that subject—1927). At an early stage of these studies, he was forced to the conclusion that the immediate, localized red reaction, followed by an oedematous wheal, which was caused by a variety of stimuli mildly injurious to the skin, was due to a chemical agent which somehow appeared as the common result of such stimuli. He inquired whether I could suggest a substance which could produce such an effect. With Sollmann and Pilcher's observations, as well as our own, freshly in mind, I supplied him with some histamine, and he found, indeed, that histamine, appropriately applied, reproduced in detail the effects in which he was interested. With exemplary caution, however, he never allowed himself to go nearer to an identification of the natural agent of the mild injury reaction than to call it "H-substance."

Histamine and Anaphylaxis. The early conception of the anaphylactic shock, as secondary to the digestive production of an anaphylatoxin in the blood, had been greatly weakened by physiological analysis of the phenomenon. Manwaring had shown that, in the dog, the syndrome was in the main, if not exclusively, the result of a primary action of the antigen on the liver. Such effects on the circulation as were not directly due to the sudden rise in portal pressure and acceleration of a substantial part of the blood in the viscera were clearly due to some vasodilator agent escaping from the congested liver into the general blood stream. Even the congestion of the liver and bowels were due, as we now know, to a substance causing constriction of the hepatic venous outlet, as histamine does. Then it was shown by Schultz (1910, 1912) that isolated plain muscle of the small intestine from an anaphylactic guinea pig responds by contraction to the direct application of the antigen. In experiments which I had begun independently, using the isolated uterus of such guinea pigs, I proceeded to examine this phenomenon in greater detail (1913), showing its close specificity and the possibility of reproducing all the characteristic phenomena of anaphylaxis in the guinea pig, active and passive, on preparations of isolated plain muscle from that species. There was a possibility, which must have been in many minds, that the union of antigen and antibody somehow led to that sudden appearance of histamine, whether in the dog's liver or the guinea pig's plain muscle. However, we had no evidence yet as to how that might happen and, as I pointed out at that time, the aggregating reaction of antigen on antibody might, on the other hand, merely provide us with a model, visible in a test tube, of the kind of action produced by histamine in the living cell.

I think that it was Lewis again who first gave public expression to the idea that an anaphylactic reaction, as seen in the skin, was, like other responses to mild injury, due to the appearance of H-substance. At that time I was busy, with the late Harold Dudley and with Best and Thorpe (1927), in a more critical confirmation and extension of my own early observations with Barger and the later ones of Abel and Kubota (1919), isolating histamine itself as a natural constituent of any organ which yielded an extract having this type of action—liver, lung, muscle, spleen—and in quantities adequate to account for such activities. Thus, we were able to tell Lewis that we believed his H-substance to be histamine itself, and that we believed its appearance, in the anaphylactic or other injury reaction, to be due to the liberation of preformed, but normally bound and inactive, histamine from the injured cells and not, as Lewis had supposed, to its new formation. Lewis and Harmer (1927) and Harris (1927) then found direct experimental evidence for this view.

Thus, the relation of histamine to the anaphylactic shock started to become clear. It was made more definite by the direct evidence, produced by Gabauer-Feulnegg *et al.* (1932), of its release in the dog's liver, and by Bartosch *et al.* (1933), of its release in the guinea pig's lung, by contact of the sensitized cells, in either case, with the specific antigen. Keeping in mind the fact that there are other physiologically active components normally held, bound and inactive, in living cells, which are likely to be similarly released by anaphylactic or other cellular injury—the adenosine series, heparin, and probably others—we begin to get a reasonable picture of the anaphylactic reaction and other consequences of extensive tissue injuries and of the part played by histamine in the resulting syndromes.

That, however, does not complete the story. In 1936, Schild suspended an isolated horn of the uterus of a sensitized guinea pig and applied histamine to it in a concentration causing maximal tonic contraction. He waited until, with histamine still present, the smooth muscle eventually relaxed from this maximum to a condition of intermediate tone with a strong rhythm. A further addition of histamine to the bath then had no effect, or even caused a relaxation. An addition of the specific antigen, on the other hand, caused the sensitized uterus to contract again to the maximum, while the effect of adding more histamine was still absent or even reversed. There are, perhaps, similar discrepancies revealed by the action of some of the antihistaminic agents. Dr. de Beer, of the Research Laboratories of Burroughs Wellcome & Company, New York, sent to me, shortly before I left England, some records obtained by Mr. Castillo with a preparation consisting of a chain of isolated rings from the trachea of a sensitized guinea pig. This gives, as expected, a contraction in response to the specific antigen, the record of which is closely similar to that of one produced by histamine; but, whereas the histamine contraction is readily relaxed by benadryl, that due to the antigen is much more resistant, relaxing but slightly in response to the same agent.

An anomaly of a somewhat analogous kind was reported by Kellaway. It was known already that the rat shows a low degree of anaphylactic sensitization and that, on the other hand, the same species is highly resistant to histamine. The plain muscle of the female rat's uterus responds to ordinary doses of histamine by a weak inhibition of its tone. Kellaway found, however, that an isolated horn of the uterus from an anaphylactic rat, while responding thus to histamine by inhibition, responded to the sensitizing antigen by an augmentation of tone which, while relatively weak, was nevertheless regular and definite. It would be of interest to know whether anything can be extracted from the plain muscle of the rat's uterus, the release of which by the anaphylactic disturbance could be responsible for a contractile response of the type observed, and, if so, whether this can be identified with any known tissue constituent. From what we know of the type of the weak response of the rat's plain muscle to histamine applied from without, we have at least no warrant for expecting, in this case, that the release of histamine in the contractile cells by an immune reaction would act as an effective stimulus.

Now, I should deprecate equally any tendency, on the one hand, to evade or slur the significance of discrepancies such as these, or, on the other hand, to regard them as immediately neutralizing all the other evidence and proving that histamine, after all, has no concern with the anaphylactic reaction in the species in which it reproduces so much of that phenomenon. Schild (1936) himself has shown that, when an isolated uterus from a sensitized guinea pig is treated with the antigen, histamine in recognizable significant amount is liberated. We have already noted the likelihood that other active constituents, normally fixed in the cell, are liberated with it. One or more of these latter may be concerned; but I am not sure that we shall need to invoke them in all these cases. Experience in another field of physiological inquiry has long convinced me that, when we are dealing with antagonisms, wide differences may be encountered between effects which are otherwise closely similar, according to whether a pharmacodynamic agent reaches the responsive cell by diffusion from without or by liberation in intimate relation to, perhaps actually within, its limiting membrane.

It has been known for many decades that, when the chorda tympani is stimulated, atropine readily stops its secretomotor, but not its concomitant vasodilator, effect on the submaxillary gland. I do not think, however, that anyone today will doubt that both effects are transmitted by the peripheral liberation of acetylcholine, presumably in relations of different intimacy to the responsive cells. Similarly, I do not think it will now be argued that vagus impulses cause contraction of the stomach wall by liberating acetyl choline and of the intestinal wall by a different mechanism, because the former effect is fairly sensitive to blockage by atropine and the latter hardly at all. Although, then, we may keep our minds still open to the possibility that all the resemblances between the effects of histamine and the symptoms of the anaphylactic reaction in different species may be misleading, I do not think that the evidence necessitates, or even justifies, any such conclusion as yet. We do not know whether the effect of histamine, when released by an aggregating immune reaction in the protoplasm of a sensitive cell, will be as readily blocked by an antagonist or by histamine itself already present in external excess, as that of additional histamine reaching the same cell by diffusion from the outside. So far as argument from analogy is justified, we should not expect the antagonism to be as effective in the former as in the latter case. And the possibility of such contrast may have an interest for the main subject of this monograph. It would not be surprising, for example, to hear evidence that an antihistaminic remedy is less effective against a bronchial spasm than against a vascular reaction in skin or mucous membrane.

Summary

Though histamine is a stimulant of nearly all plain muscle, the intensity of its action is not uniformly distributed. The plain muscle coats of the bronchioles and of the uterus, especially in the guinea pig, seem to be especially responsive to its action. That of the guinea pig's ileum is also exquisitely sensitive. This, with its relaxing effect on capillary walls, seems to account well enough for the manifestations of its actions in the asthmatic and urticarial reactions of the allergies. Additional effects, not obviously connected with these two main actions, are the stimulation of gastric secretion, the production of severe headache, and a rapid fall of the body temperature. Apart from its release by allergies and other injurious reactions, it is released, in quantities sufficient to produce general vasodilator reactions and gastric secretion by certain substances acting as "histagogues," by curarine (Alam, Anrep, Barsoum, Talaat, and Wieninger, 1939) and certain diamidino and diguanidino derivatives as well as by licheniformin, an antibiotic (MacIntosh and Paton, 1947).

There remains a question of great physiological interest: whether and, if at all, to what extent the vasodilator action of histamine participates in the functional adjustment of the circulation to metabolic need. Since Gaskell (1877) first put forward the suggestion seventy years ago, there has accumulated a large volume of evidence for a predominant role of substances liberated from the cells of a tissue during active function, in the dilatation of its blood vessels, often long outlasting the phase of activity. The substances concerned have often been spoken of collectively as "metabolites"—a term which is unsatisfactory, not only as being vague, but as implying, at least in advance of the evidence, that the substance or substances in question are actually products of, and not merely that their appearance is a concomitant of, the accelerated metabolism.

In the Herter Lectures (Dale, 1920) delivered on the occasion of my first visit to the United States, I discussed in some detail the possibility that histamine might at least participate in the production of this functional hyperaemia. I pointed out that the predominant incidence of its vasodilator action on the smallest blood vessels, and particularly on the capillaries, should render it an ideal agent for the fine adjustment of the circulation to varying, local, metabolic demands, enabling it to shift the prevailing course of the blood flow from one mesh to another of the capillary network as one cell or tissue unit after another became involved in activity. I was obliged, however, to admit that such speculations would have no basis but imagination, unless we could obtain direct evidence to justify the assumption that histamine is, in fact, liberated from cells which enter into functional activity.

The position has changed in more than one respect in the 29 years which have intervened. We know now that most, if not all, of the body cells which exhibit a vigorous metabolism do contain histamine as a normal constituent of their protoplasm. We know also, especially from the work of Lewis and his school, that stimuli applied to such cells, if they are vigorous enough to entail even mild injury, do, in fact, cause this histamine to be liberated from them in sufficient amounts to cause dilatation of the adjacent capillaries, to a degree which can be regarded as mildly pathological. For direct evidence of the liberation of histamine by normal cellular activity, in a manner adequate to account for functional vasodilatation, we seem as yet to be limited to the highly suggestive experiments of Anrep and his co-workers. Anrep and Barsoum (1935) and Anrep, Barsoum, Talaat, and Wieninger (1939) found that contraction of voluntary muscle in the dog, whether evoked by artificial stimulation of the nerve or by centrally convulsant drugs, in the normally innervated muscle, or by direct stimulation of the muscle denervated by degeneration, is accompanied by an output of histamine, appearing in the venous blood from the muscle as long as the hyperemia continues, which is evoked by and continues after the contractile activity.

The experimental findings presented by Anrep and his colleagues appear to be so clear cut and regular, and their significance, if established beyond question, seems likely to have wider applications of such importance that it is to be hoped that confirmation and extension of them may soon be forthcoming from other laboratories. If the functional liberation of histamine is to be accepted, the question still remains whether it acts by itself or by a synergism with CO_2, a possibility suggested by observations recently shown to me by H. Rein, in Göttingen. Further, and as a point which seems to me to have special relevance to the subject of this monograph, if histamine does in fact, and to any degree, play an essential part in such a fundamentally important physiological mechanism of adjustment, we must surely keep that function in mind in considering the actions and the desirable applications of the antihistaminic remedies. We ought, perhaps, to seek some assurance that the use of these substances to give relief from distressing and deleterious symptoms, due to pathologically liberated histamine, does not entail the harmful suppression of a part played by histamine in a normal and essentially physiological adjustment. One should hesitate, in default of direct and compelling evidence, to proceed on the assumption that the natural occurrence of histamine, in the active cells of so many organs and tissues, has none but a potentially injurious significance.

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THE PHARMACODYNAMICS OF HISTAMINE

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To make understandable the case history, as it were, of allergy, one must be familiar with the characteristics of the "skeleton in the closet." Histamine plays the role of such a "skeleton" in relation to allergy. Since the papers dealing with the peculiar problems of allergy have clarified this relationship, we shall confine ourselves purely to the characteristics of that "skeleton," save for minimal references to the earliest papers suggesting a connection between allergy and histamine.

Histamine was first synthesized in 1907 by Windaus and Vogt.[1] In 1909, Biedl and Kraus[2] advanced the hypothesis, based upon their studies of peptone-shock and anaphylaxis, that the symptoms resulting therefrom might be attributable to the release of a toxic substance within the organism. In 1910, Kutscher[3] and Barger and Dale[4] announced their independent isolation of histamine from ergot. In the same year, Dale and Laidlaw,[5] after a careful study of the physiological effects of histamine, suggested that this substance might be responsible for the symptoms of peptone-shock. The comprehensive investigations of Dale and Laidlaw concerning the physiological effects of histamine clearly delineated the major actions of the drug.

The hundreds of papers concerned with histamine that have been published since that time have added details to the picture sketched by Dale and Laidlaw. We can consider the present state of that picture, but it would be impossible to trace the history of every detail. Such histories are readily available in the two able monographs by Feldberg and Schilf[6] and by Gaddum and Dale[7] and in the four excellent reviews by Best and McHenry,[8] Rose,[9] Dragstedt,[10] and Selle.[11] Of the many investigations in recent years that have been concerned primarily with the metabolism of histamine, only a partial review will be possible.

Histamine is readily absorbed after injection and rapidly exerts its effects. Histamine administered enterally is slowly and poorly absorbed, although slight absorption does occur from the small intestine.[12] Histamine must be reasonably well absorbed from the oral cavity, since, when administered lingually, it has been lethal to guinea pigs.[13]

Best and his associates[14] first definitely established the presence of histamine in most, if not all, tissues of the body. Earlier workers had isolated histamine from various tissues,[15] but the demonstration by Ackermann[16] in 1910 that bacteria could produce histamine from histidine had raised the question of whether the histamine subsequently isolated was a natural constituent of the tissues or was produced by decomposition. The analytical method devised by Best and his associates eliminated this doubt. In general, its concentration is particularly high in lung, intestine, and skin and usually particularly low in the blood and kidneys; but there are considerable variations among species.

In 1935, Barsoum and Gaddum[17] devised a more sensitive analytical procedure. It still employed, however, essentially the same principles incorporated in the method of Best and his associates. Code[18] added to their procedure minor changes that served to make it more practicable. Using his modified procedure, Code and others have confirmed the earlier results.

Histamine injected intravenously rapidly leaves the blood stream.[9] The reason for this is not known, since, to date, the only enzyme specifically inculpated in the detoxification of histamine is histaminase, first isolated by Best and McHenry[19] and later studied by them in detail. The existence of such an enzyme had been foreshadowed by the report of Eustis[20] in 1915 that histamine was inactivated after incubation with buzzard's liver. This enzyme has since been shown, by Zeller and his associates,[21] to be a relatively unspecific diamine oxydase. This enzyme acts *in vitro* very slowly, whereas the detoxification of histamine *in vivo* is very rapid. This suggests one of two possibilities: (1) either other enzymes are concerned with the detoxification of histamine *in vivo*; or (2) there are present, *in vivo*, substances that markedly accelerate the action of histaminase.

Rocha e Silva[22] has suggested that histamine may be temporarily inactivated by combination with the free carboxyl groups of amino acids within the cells. There is also evidence that certain non-specific detoxifying agents, such as ascorbic acid, glycine, glucuronic acid, and cystine, may play a role,[23] a possibility strengthened by the report that the administration of histamine would increase the urinary excretion in guinea pigs of combined ascorbic acid.[24] Some histamine is excreted by way of the urine, from which it was isolated by Ackermann and Fuchs.[25] Anrep and associates[26] showed that it is excreted in both a free and combined form, with the combined form predominant in Carnivora and the free form in Herbivora. The administration of histidine has been reported, by Holtz and Credner,[27] to increase the urinary excretion of histamine. This is reasonable, since histidine by decarboxylation within the body gives rise to histamine. Alexander[28] reported that urinary excretion in mice tended to parallel the total concentration of histamine in the body. It is certainly clear that urinary excretion is not a major method by which the body eliminates excessive histamine. Histamine has also been isolated from feces by Myhrman,[29] from menstrual fluid by Gibertini,[30] and from human milk by De Toni.[31]

The problem of tachyphylaxis and tolerance to histamine has been too recently reviewed by Wells, Gray, and Dragstedt[32] to justify its repetition. They found no evidence for either phenomenon, and, in several hundred experiments in our laboratories with dogs with Thiry-Vella loops that received many injections of histamine at rather frequent intervals, we have seen neither phenomenon. Cantoni[33] recently reported that the tachyphylaxis to large doses of histamine originally reported by Barsoum and Gaddum[17] can be eliminated in *in vitro* experiments by increasing the concentration of potassium in the perfusion fluid. One cannot deny that others have found evidences of both tolerance and tachyphylaxis. It is obvious that the conditions of the experiments could not have been the same. It re-emphasizes the need for standard conditions in investigating the phenomena of tolerance and tachyphylaxis.

Three main physiological actions account for most of the effects of parenterally administered histamine: (1) its stimulating action upon smooth muscle; (2) its dilating action upon capillaries, which is extended in some species to arterioles (other portions of the vascular tree are, in general, constricted); and (3) its stimulating action upon glands, including the gastric glands and the adrenal medulla.

The stimulating action upon smooth muscles includes almost all those that might be mentioned—the bronchial muscles, intestinal, splenic, vesical, those of the gall bladder, small intestine and colon, uterus, vas deferens, and epididymis.

The sensitivity of various organs and of the same organ in different species differs greatly. These wide variations in sensitivity, as well as certain anomalous results, present challenging problems to a solution of which our present knowledge is unfortunately unable to contribute. Thus, the lapin ileum responds, *in vitro*, only to a dose several hundred times that needed to cause an excellent contraction of the ileum of the guinea pig. The uteri of all species studied are contracted by histamine, save that of the rat, which is relaxed. A more extensive list of these peculiarities would be uninspiring and useless, since any principle that would permit correlation has thus far eluded us.

The action of histamine upon the blood vessels accounts for many of the symptoms that its injection can produce. The capillaries are dilated, a presumed direct effect unaltered by atropine. Knox,[34] however, recently reported that the enhanced dilatation caused by histamine of the vessels in the leg of the cat perfused with a calcium-free fluid could be abolished by atropine. The arteries and veins are contracted by histamine. The action upon the arterioles varies with the species and tends, in the highest mammals, to be dilatation and, in the lowest mammals, to be constriction. Once again, we encounter unexplained exceptions. Thus, Woodbury and Hamilton[35] concluded that histamine did not constrict the pulmonary vessels of the dog as it did in the cat and rabbit, and the experiments of Friedberg, Katz, and Steinitz[36] confirmed their results. The net effect of histamine in any species upon the blood pressure, which is the algebraic sum of many variables, will depend considerably upon the relative sensitivity of the various portions of its vascular system. Thus, in man and monkey, whose capillaries and arterioles are dilated by histamine, a hypotension results from the administration of the drug; whereas in the rabbit, whose arterioles are constricted and only the capillaries dilated, a hypertension is seen.

Histamine has a significant stimulating action upon many glands. Dale and Laidlaw[5] first remarked this in relation to the lachrymal, bronchial, salivary, and pancreatic. The remarkable stimulating action of histamine upon the gastric glands was first observed by Popielski[37] in 1920. This has since been intensively investigated. The controversy, as to whether gastric secretin is histamine, has not yet been satisfactorily settled. The difficulties that must be resolved in order to answer this problem have been discussed by Emmelin,[38] who favors the view that gastric secretin is not histamine, but depends for its action upon a liberation of histamine in the gastric mucosa. Ackermann,[39] in a careful review of the physiology and pharmacology of the sweat glands, concluded that histamine had little effect thereon. The action of histamine upon glands presents many interesting problems which, unfortunately, we do not have the space to consider.

The observations of Gibbs and McClanahan,[40] that atropine would inhibit the sialogogic effect of histamine, has been confirmed many times. Cornil[41] reported that, in the curarized dog, the stimulation of histamine upon the salivary glands was more pronounced. This recalls the observation by Alam, Anrep, Barsoum, *et al.*[42] that curare caused a relatively marked increase in the blood histamine.

Injections of histamine have been reported to cause an increase in the pressure of the cerebral spinal fluid. Such a rise was reported by Weiss and his associates[43] and was later confirmed by Friedman[44] and others. A similar response was reported for the dog. Recent experiments in our own laboratory, however, in which the cerebral spinal fluid pressure has been continuously recorded, have indicated that one may see a rise, a fall, or a biphasic reaction in which an initial fall is followed by a secondary rise. The last reaction is the most common. These variations are not surprising when one considers that the cerebral spinal fluid pressure is as much the algebraic sum of many variables as is the blood pressure. The initial fall probably represents a decreased circulation to the brain as a result of the sudden hypotension; a secondary rise probably reflects the secondary cerebral vascular congestion; and the slow but continuous rise that may be seen in some experiments after repeated doses of histamine may reflect some increase in the permeability of the choroid plexus.

The actions of histamine upon the heart are of interest, although the results reported are not entirely in accord. Lissak and Kokas[45] reported that histamine had a positive inotropic effect on the isolated spring frog heart, with a negative effect upon the winter frog heart. He attributed this to the level of metabolism, since thyroidectomy of the spring frogs reversed the positive inotropic effect. These effects were confirmed by Martin.[46] The effects of histamine upon the human heart were studied with electrocardiograms by Massione and Picchio,[47] by Weiss and his associates,[48] and more recently by Albers and Petzold.[49] They concluded that the abnormalities observed reflected significant toxic effects. Peters and Horton,[50] however, made similar studies during a constant infusion of histamine and considered the electrocardiographic changes, the most common of which was the loss or inversion of the T wave, of minor consequence, especially since they disappeared shortly after the discontinuance of the infusion. Hueper and Ichniowsky[51] made extensive pathological studies of dogs that had survived prolonged and severe histamine shock. They found serious and irreversible changes in the myocardium, as well as in many other tissues. These changes were probably not direct effects of the drug but were rather the results of the shock induced by the histamine

The lymphogogic action of histamine, initially reported in dogs by McCarrell and Drinker,[52] has since been confirmed by several other investigators. This has been considered secondary to the general vascular changes, in particular, the increased pressure in the portal system.

The relation between the adrenals and the physiological actions of histamine has received such intensive study that it would be impossible to attempt more than a summary of the results. In 1920, Dale[53] reported evidence that histamine increased the output of the adrenal medulla, but it was not until 1926, when Burn and Dale[54] were able to show that the secondary rise in blood pressure in cats following the injection of histamine could be eliminated by removal of the adrenals, that definite evidence for the relationship between the two was available.

In 1926, Banting and Gairns[55] first demonstrated that, in dogs, adrenalectomy caused a remarkable increase in the animals' sensitivity to histamine. Wyman, in 1928,[56] confirmed this with rats and showed that their sensitivity could be decreased by an intraperitoneal injection of epinephrine. Perla and Gottesman[57] were not able completely to protect their rats by epinephrine. In 1937, Ingel[58] sought to restore the resistance of adrenalectomized rats to histamine by the administration of cortin, but he was unable to do so until the cortin had been supplemented by epinephrine. Rose[9] has shown that the rat's capacity to detoxify injected histamine is impaired by adrenalectomy. He has also shown that very large doses of cortin would decrease the rat's sensitivity to normal levels. It would appear from these and other results that the increased sensitivity of adrenalectomized rats to histamine is due to a loss of both secretions of the adrenals. The recent report by Staub[59] that therapeutic doses of epinephrine injected into patients immediately and briefly increases the blood histamine is most interesting, but serves, at the moment, to increase the problems of interpretation. Burn and Dale[60] had suggested such a possibility, but Dale and Richards[61] were unable to demonstrate it. It remained for Eichler and his associates[62] to prove it occurred, although they employed nonphysiological doses in cats (as high as 3.0 mg/kg).

Thus far, attempts to explain the normal functions of histamine within the organism have been largely unsuccessful. Its marked oxytocic action has tempted efforts to inculpate it not only in the onset of normal labor, but in the toxemias of pregnancy as well. Such studies were initiated by Kapeller and Adler[63] and have been extended by Werle and Effkemann[64] and others. They have indicated that the blood content of histaminase increases in normal pregnancies, and that, in pathological pregnancies, abnormalities in the metabolism of both histidine and histamine can be detected. The significance of these changes remains obscure. Kwiatkowski[65] has presented evidence that nervous stimulation may cause a release of histamine in certain tissues. This would provide us with a class of histaminergic nerves. Rosenthal and Minard[66] advanced the theory that histamine was the chemical mediator for nerves transmitting sensations of pain. The marked local anesthetic action of the antihistaminics now clinically employed offers indirect support for their hypothesis. Whether the antihistaminic and local anesthetic actions of those drugs are separate, unrelated attributes or merely divergent expressions of the same fundamental action has not yet been determined.

It has been shown by Anrep and his associates,[67] Ambache,[68] Marcu,[69] and others that all three types of muscles—skeletal, cardiac, and smooth—liberate histamine during activity. No explanation is available for this interesting but as yet unconfirmed finding. The problems of the physiological functions of histamine must await further research for solution. It is certain that histamine must fit into something more than a theory of allergy.

In conclusion, a brief discussion of the mechanism of histamine's action would appear relevant. Histamine has been classically considered a myotropic stimulant. Somewhat over two years ago, in our laboratories, a study was initiated of the relative potency of various antihistaminics in nullifying the intestinal spasm induced by injected histamine in dogs with Thiry-Vella loops. Anomalous responses to histamine began to appear which the classical theory could not explain. Early in 1946, Ambache[70] advanced the hypothesis and offered persuasive evidence therefor that histamine acts by causing a liberation of acetylcholine within the body. Since this hypothesis explained the peculiar results we had obtained, we sought to repeat his experiments in part and to test the validity of his hypothesis by other methods.

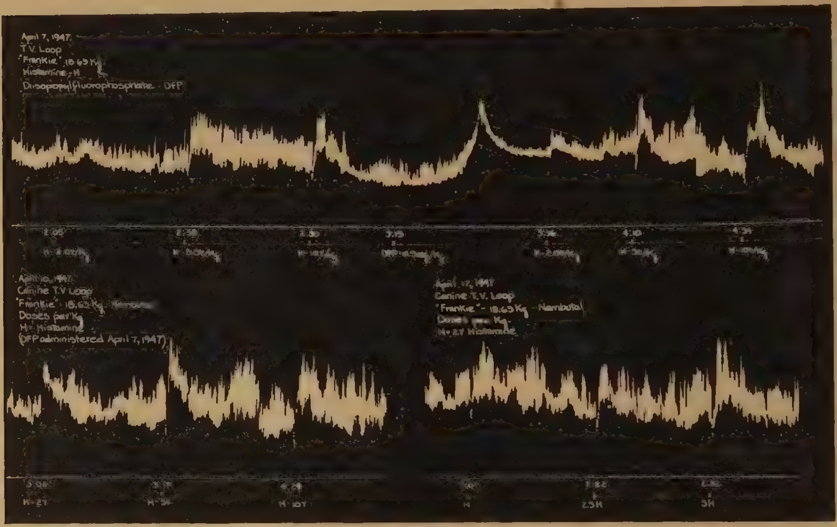

FIGURE 1. *April 7, 1947.* In this record the stimulating action of di-isopropylfluorophosphate upon the intestine is evident, as is also its potentiation of the action of subsequently injected histamine. The lower left-hand record, secured in an experiment on the same dog performed three days after the injection of DFP, shows some persistence of the potentiating action. In the lower right-hand record, secured in an experiment performed on the same dog, ten days after the injection of DFP, the potentiating action is lacking.

We attempted to demonstrate that sections of the small intestine of the rabbit or cat would, after a variable number of days of cooling in Locke's solution, attain a state in which they had lost all responsiveness to histamine but would still respond to relatively large doses of acetylcholine. Ambache had demonstrated such a state with the intestines of the rabbit and guinea pig. His explanation thereof was that the tissues had lost their capacity to produce acetylcholine and hence would no longer react to histamine, the effect of which depended upon an endogenous release of acetylcholine.

Since the muscular mechanism remained at least partially functional after the degeneration of all nervous tissue, added acetylcholine still worked.

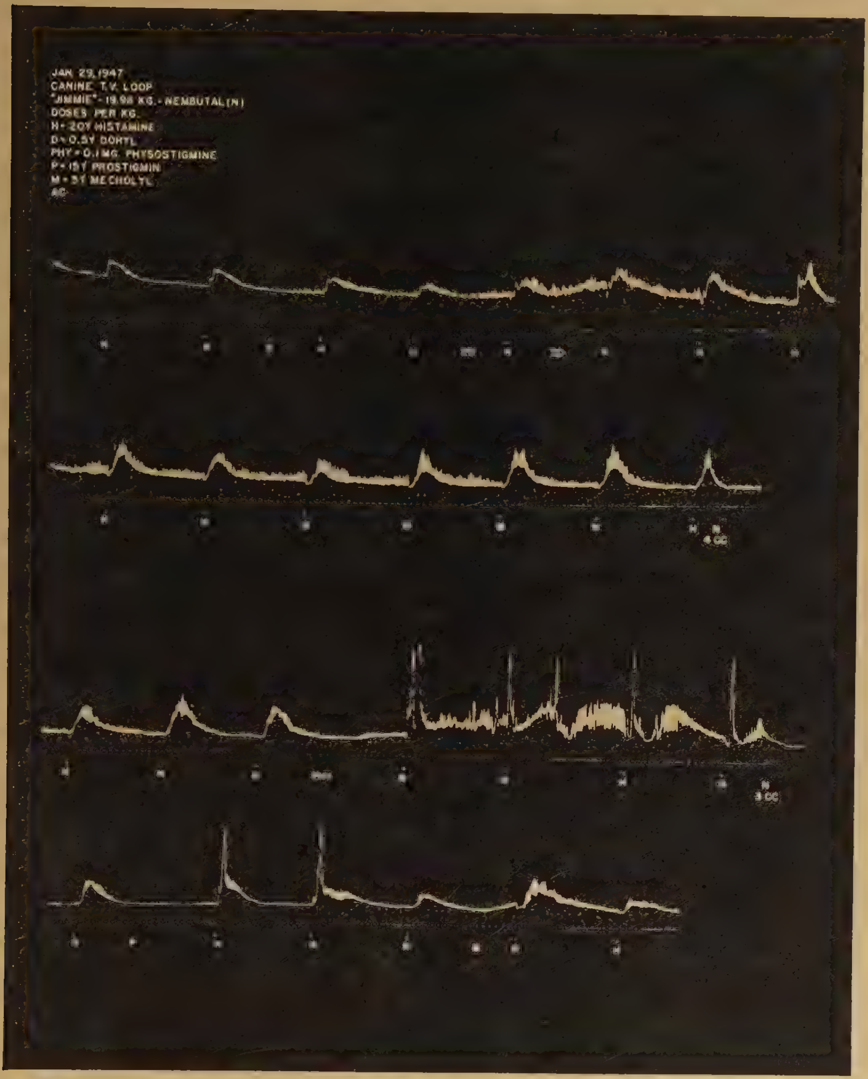

FIGURE 2. *January 29, 1947.* The marked potentiation of histamine's action by physostigmine and prostigmine is evident. Doryl and Mecholyl may be seen to have caused little or no potentiation.

Unfortunately, in our own experiments, we found the responses to histamine and acetylcholine disappeared *pari passu.*[71] Although conditions for our experiments were similar, thus far, the cause of the discrepancy has eluded us. It was possible to show that the intestinal spasm induced in Thiry-Vella loops by intravenously injected histamine could be potentiated by di-isopropylfluorophosphate, physostigmine, and prostigmine, the actions of which depend primarily at least upon an inhibition of cholinesterase, but not by Mecholyl or Doryl, the chief mode of action of which is different. The histamine-induced intestinal spasm was accentuated and prolonged in adrenalectomized dogs. This was expected, because histamine has been shown, since the first suggestion by Dale in 1920,[72] to increase the adrenal's output of epinephrine, whose actions antagonize those of histamine. This effect might be due to a release of acetylcholine at the preganglionic neural endings within the adrenal medulla.

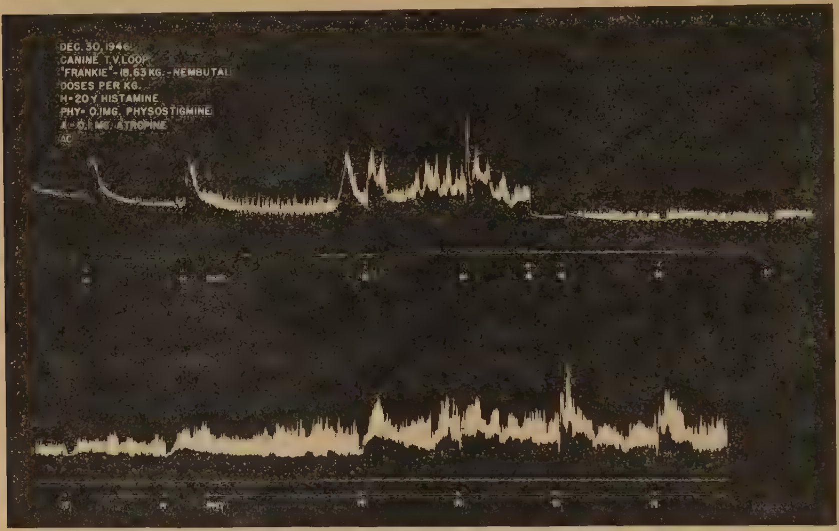

FIGURE 3. *December 30, 1946.* The record reveals the bi-phasic nature of the intestine's response to histamine. After atropine, the stimulating action of histamine is absent and its inhibitory action marked. The antihistaminic activity of atropine may be seen as well as the potentiation of histamine's action by physostigmine.

The action of histamine upon the intestine was shown to be biphasic with an initial, and at times scarcely discernible, inhibition which was quickly followed by marked stimulation. After the administration of atropine, the stimulation was absent and the inhibition more marked. A possible explanation might be the liberation of acetylcholine at preganglionic sympathetic endings, since atropine is considered to negate the effects of acetylcholine only at the post-ganglionic endings of the parasympathetic nerves.

These data strengthen Ambache's hypothesis, but it is by no means as yet a theory. It is certain that the pharmacological actions of acetylcholine and histamine are very similar. There are, however, disturbing discrepancies. To mention but two: (1) acetylcholine contracts the uterus of the rat, but histamine does not;[73] (2) acetylcholine dilates arterioles, but histamine generally constricts them.[9] It has become increasingly apparent that intimate relations exist amongst the three powerful physiological agents acetylcholine, epinephrine, and histamine. The future yet holds the interesting day when their biographies become completely known.

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THE ROLE OF HISTAMINE AND OTHER METABOLITES IN ANAPHYLAXIS

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To discuss the role of histamine and other metabolites in anaphylaxis as a part of this monograph is to risk the Scylla of a burdensome repetition of an oft-told tale and the Charybdis of inexcusable neglect, since the principal topic, "Antihistamine Agents in Allergy," traces its genealogy to this experimental problem. I shall try neither to bore you with all the oft-quoted evidence for the role of these agents in anaphylaxis nor to evade my responsibility in clarifying the background for this monograph and the corresponding background for the current approach in the therapy of allergy.

The prevalent theory as to the pathogenesis of the varied symptoms appearing during anaphylactic shock holds that these symptoms are mediated by histamine and other tissue metabolites which are liberated in some manner as a consequence of the antigen-antibody reaction. If this is viewed as a theory, subject therefore to amendment, support, or refutation, it seems not inappropriate to survey briefly the various theories which preceded it, for the *post-mortem* analysis of deceased theories serves to emphasize the criteria by which we may assess the fraility or strength of a current postulate.

There are a number of theories that require no more than mention, for the assemblage of facts which has destroyed their tenability is well known to all. Among such are the theories that the anaphylactic symptomatology may be ascribed (1) to an excitation of an immunity center in the central nervous system, (2) to intravascular coagulation and embolism, (3) to esoteric disturbances in the colloidal balance of the circulating plasma, (4) to altered viscosities in the blood and muscle plasma, (5) and to the toxic half of the antigen, envisaged as a sort of Siamese-twin union of toxic and nontoxic units.

Worthy of more extended mention, of theories respecting the mechanism of the anaphylactic reaction, was the crystallizing of ideas into what were called the anaphylatoxin or humoral hypothesis, on the one hand, and the cellular theory on the other. Taking origin in, or at least a hint from, Pfeiffer's original endotoxin theory as to the pathogenesis of symptoms in infectious diseases, and receiving varying kinds of support from the observations of Wolff-Eisner, Friedberger, Vaughan, and Wheeler and others, the general tenor of this anaphylatoxin hypothesis was to the effect that anaphylactic shock is a true intoxication due to a poison produced from the products of the antigen-antibody reaction by the action of complement, the reaction occurring intravascularly.

Contrariwise, the observations of Manwaring, Pearce and Eisenbrey, Coca, Weil, Schultz, Dale, and others appeared, presenting such unequivocal support to the rival cellular theory that, in 1923, Zinsser summarized the situation as follows: "There is, thus, an incontrovertible mass of evidence available which proves without question that the site of the reaction, which carries in its train the symptoms which we speak of as anaphylaxis, is predominantly on the cells of the body."

This verdict, in view of the irreconcilability of the opposing theories, was, thus, a death sentence to the anaphylatoxin hypothesis. This was regrettable in that the anaphylatoxin hypothesis, in addition to speculating as to the site of the reaction, presented an intelligible explanation of the ensuing phenomena, while the cellular theory was at that time restricted to localizing the site of the reaction and left largely to the imagination the mechanism by which a cellular reaction could induce the characteristic phenomena of anaphylaxis.

Meanwhile, the observations of Dale and Laidlaw on the pharmacological effects of histamine, of Barger and Dale and of Best, Dale, Dudley, and Thorpe on the presence of histamine in animal tissues, and of Lewis on various human skin reactions, paved the way for Dale's extension of the cellular theory, which, although phrased in 1929, is still such a cogent statement of our current view that it demands repetition. He said, "We may picture the anaphylactic shock, therefore, as the result of cellular injury, due to the intracellular reaction of the antigen with an aggregating antibody. Whether this is general or localized in a particular organ, histamine will be released and its effects will be prominent in the resulting reaction, imposing a general resemblance to the syndrome produced by histamine itself, on the symptoms seen in each species. The cell injury, however, is not limited to the degree required to produce a release of histamine, and involves other and more direct results. Such a conception is in accordance with all the facts as yet available, and it has the advantage of rendering intelligible, not only the striking resemblance between symptoms of the anaphylactic reaction and those produced by injecting histamine, but also the various and equally significant points of difference between the two syndromes."

In 1932, however, after the first experimental evidence directly supporting Dale's theory had been reported, an editorial commentator wrote as follows: "However, the existence of a secondary allergic toxic factor can no longer be doubted if one is to credit the current biochemical studies by Dragstedt and Gebauer-Fuelnegg of Northwestern University Medical School, and the simultaneously reported anaphylatoxin studies by Bartosch, Feldberg, and Nagel of the physiologic institute at the University of Berlin." This maladroit employment of the term "anaphylatoxin," with reference to our observations on the release of histamine in anaphylaxis, seemed to imply that there was an attempt at a reincarnation of the defunct anaphylatoxin hypothesis and thereby an attempt to discredit the cellular theory, for, as Zinsser had concluded, "All workers who have inclined to an anaphylatoxin theory have necessarily assumed the site of the reaction to be in the circulation."

Except for the borrowing of this euphonious term from the opposing anaphylatoxin theory, there is no conflict whatsoever between Dale's histamine theory, with the experimental evidence supporting it, and the so-called cellular theory. Dale's statement and all of the experimental evidence acquired during the past fifteen years are only extensions of the cellular theory and have clothed the cellular theory with meaning and verified its validity. This point cannot be stressed too forcefully, because I am convinced that almost all the opponents of the histamine theory base their opposition upon a misunderstanding of this fact. For example, a recent reviewer writes as follows: "Some of these objections, however, have been adequately met by more recent findings; others have not, and until they are all satisfactorily answered, the histamine hypothesis cannot take precedence over the cellular theory."

Viewing the anaphylactic reaction as a special form of cellular injury which permits the release of cellular metabolites, we are concerned with those metabolites which, by virtue of having physiological activity, might impose their effects upon the diverse pattern of the reaction. The list would include adenosine, acetylcholine, heparin, histamine, lysocithin, potassium, various intracellular enzymes, and probably others.

The problem of assessing the role of these agents in the anaphylactic reaction becomes one of cataloguing the anaphylactic symptomatology, identifying one or more of these metabolites in a causal relationship to one or more symptom, and determining how well we can balance the accounts. Of paramount importance is the necessity of a quantitative evaluation. One of my late professors always cautioned his students about the fly that sat on the axle of the speeding chariot wheel and bragged of the dust he was raising. Thus, the mere recognition of one of these metabolites in association with the anaphylactic reaction does not distinguish it as a participant rather than a spectator in the performance, although one would have to grant that its peculiar properties, viewed in the light of the nature of the anaphylactic reaction with which it was associated, might give good grounds for suspicion.

Probably the clearest case of all has been made with respect to the role of heparin. Since Jaques and Waters identified, isolated, and quantitated heparin in the blood of anaphylactic dogs, there has been no question as to its role in the incoagulability of the blood in this animal. Although incoagulability of the blood is a less conspicuous phenomenon in the anaphylactic reaction of other animals, there is good ground for assuming that it might account for such changes as do occur. Parenthetically, it seems odd that there have been no studies reported, so far as I am aware, as to whether heparin does, or does not, appear in human blood during severe allergic reactions. Correspondingly, it seems odd that the opponents of the histamine theory have not had recourse to considering the absence of such evidence in the human reaction either as an argument against the theory, as applied to anaphylaxis on the one hand, or as an argument against the identity of the anaphylactic reactions of animals with the allergic reactions of man on the other.

Acetylcholine, adenosine, choline, lysocithin, potassium, the slow-reacting-substance of Kellaway and Feldberg, and a proteolytic enzyme have all been identified, more or less definitely, in connection with anaphylactic reactions. The indictment of them as participating agents in the reaction has not as yet progressed much beyond the recognition that they might play some role, nor have they been fully exonerated by any evidence which would preclude their participation. At the moment, their identification serves to indicate the complex character of the cellular injury involved in the anaphylactic reaction and to suggest potential mechanisms which may explain and clarify some of the symptoms of anaphylaxis not otherwise accounted for.

With respect to histamine, its status as a participating factor in the anaphylactic reaction seems to be midway between that of all these agents, which as yet are only suspect, and that of heparin, which may be considered unequivocal. In thus abridging the status of the role of histamine, it is not intended that its importance be in any way minimized.

Consider for a moment the nature of the evidence implicating histamine. In the dog, histamine has been shown to leave the liver and to appear in the blood and lymph, both in such quantity and with such explosive rapidity as to permit no reasonable alternative to its participation in the reaction. The evidence is essentially as conclusive for the guinea pig, although the source of the histamine in this animal is more pulmonary than hepatic. Since the administration of histamine, in doses equivalent to those estimated as released in anaphylaxis, has serious to fatal effects in these animals, one cannot deny that it has a major role in the anaphylactic reactions of these animals, even though evidence to preclude the collaboration of other factors is incomplete. The role of histamine in the anaphylactic reaction of the rabbit is not so clear.

There is no doubt that histamine is released from the cellular elements of the blood into the plasma, thereby providing the opportunity for it to become physiologically active. There is no doubt that this release occurs with sufficient rapidity. As yet, however, we do not have adequate data of a quantitative character to permit an evaluation of the importance of this histamine release, and we do have some evidence that obstruction to the pulmonary circulation, independent of histamine, may have significance. Thus, the status of the role of histamine in anaphylaxis is more ambiguous than that of heparin, in that the collaboration of other agents or factors cannot be excluded so readily. Its importance, however, is far greater. The discharge of heparin does not appear to jeopardize either the life or health of the animal and it is not known to be concerned in any important phase of the reaction other than that of the coagulability of the blood. Histamine, however, is important by virtue of the serious import of its effects as well as by its ability to impose its action in such diverse fashion.

To draw up a provisional balance of accounts between the varied symptoms of anaphylaxis on the one hand and the tissue metabolite most probably concerned in its pathogenesis on the other, it is clear that heparin can account for the incoagulability of the blood and perhaps for some minor phenomena, such as the anticomplementary titer of the blood. It is also clear that histamine can account for the majority of the smooth muscle reactions, glandular secretions, increased lymph flow, and vascular phenomena. This leaves some characteristic features of the anaphylactic reaction unaccounted for, such as the necrosis of tissue as seen in the Arthus' phenomenon, *etc*. To what extent the agents other than heparin and histamine, which have been identified with the reaction, contribute to the syndrome is not yet clear.

The proponents of the original anaphylatoxin hypothesis devoted a great deal of effort to finding some unitarian toxin which would have all of the diverse effects which are manifest in the anaphylactic reaction. The opponents of the modern extended version of the cellular theory seem to have been converted to this unitarian doctrine, in that they are inclined to impeach any tissue metabolite identified with anaphylactic shock on the basis of the incompleteness with which it mimics every item in the category of anaphylactic symptomatology. As it happens, they have directed their criticism at histamine and not at all at heparin. I do not understand the logic of this attitude. That histamine may have less significance in the reaction of other animals than in that of the dog and guinea pig is of no more moment than the fact that heparin has less significance in other animals than it has in the dog. Nor, to generalize, is there good reason why the other tissue metabolites associated with the phenomena of anaphylaxis should have parallel degrees of importance in more than one animal species.

With respect to the present monograph, the role of histamine and other metabolites in anaphylaxis has significance only to the extent that the problem of human allergy has kinship with the problem of anaphylaxis and to the extent that the agents shown to be of importance in the one case are also shown to be of importance in the other. It is not in my province to discuss this question, and I shall merely comment that it seems strange that one of the opponents of the histamine theory should have written a paper in 1930 defending the fundamental identity of animal anaphylaxis and human allergy, but now, though admitting the causal relationship of histamine to anaphylaxis in the dog and guinea pig, discredits its possible role in human allergy because it has not yet been proven to be of paramount importance in the rabbit, horse, and calf. I do not believe that an argument on the basis of phylogenetic relationship is any better in one case than it is in the other.

In conclusion, I beg your indulgence for a parody on Kipling's immortal eulogy to Gunga Din:

HISTAMINE

By Carl A. Dragstedt

You may talk of toxicosis,
Migraine, shock, or halitosis—
Two to one there'll be a heckler on the scene,
Who'll report his observations,
Mingled with imaginations,
Placing all the blame on evil Histamine.

For it's 'mine, 'mine, 'mine,
By your real name or your alias, ergamine,
You give asthmatics wheezes,
Pollinosis patients sneezes—
You smooth-muscle stimulating Histamine.

Trauma, burns, and inflammation,
Headache, pain, and constipation
Show the fingerprints of some malicious fiend.
And the one that gets accuséd
Is that amine so abuséd—
Beta iminazol ethylamine.

For it's 'mine, 'mine, 'mine,
On you every research worker vents his spleen.
Though obscure as yet, the fact is
You're involved in anaphylaxis—
You capillary poison—Histamine.

Since the days of Dale and Barger,
His dossier gets longer, larger,
And the files will show some chapters in between,
Indicating he's suspected
Of some items here neglected—
That secretion stimulating Histamine.

For it's 'mine, 'mine, 'mine,
You decarboxylated son of histidin.
We've extracted you and weighed you,
By the living gut assayed you,
But we're yet to get your measure—Histamine.

1947

THE ROLE PLAYED BY LEUCOCYTES AND PLATELETS IN ANAPHYLACTIC AND PEPTONE SHOCK

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If one goes back to the earlier theories of anaphylaxis, the number of separate points of view, or schools, defending entirely independent ideas on the mechanism of this important phenomenon becomes quite impressive. We might distinguish among the different conceptions—the immunological, the hematological, and the physiological or pharmacological—according to the main principles involved and the personal interests and scientific outlook of their proponents. Today, it is rather trivial to say that the liberation of histamine plays a major role in the production of the symptoms and that other metabolites, such as adenosine, acetylcholine, a "slowly reacting substance" (S.R.S.), *etc.*, might also contribute to initiate or aggravate shock. There is also no question that heparin, as such, is released from the liver and that it could explain incoagulability of the blood, and that the opposite effect (*i.e.*, the decrease in clotting time as it has been observed in anaphylactic and peptone shock during the so-called negative phase) might depend upon activation or release of thromboplastic agents, among which one might include activation of a proteolytic enzyme of the trypsin type.

It is obvious, however, that this description does not include many other phenomena that occur in anaphylactic and peptone shock with at least an equal degree of frequency. I pass over the immunological point of view, since there is a general agreement that the whole anaphylactic reaction takes place as a consequence of a combination of the antigen with the antibody, this being a sort of trigger mechanism for the whole physiological process to develop. The fact that similar phenomena and symptoms can be obtained by injecting peptone led to the conclusion that the symptoms following combination of the antigen with the antibody are common to other forms of aggression to the cell and by no means represent the direct, toxic effect of the so-called antigen-antibody complex. Therefore, reconciliation of the physiological or pharmacological point of view with the immunological one is much easier than might appear by reading papers from either side.

But, now, let us consider the hematological point of view. First of all, I wish to mention the old French conception of the "colloidoclastic shock," which implies a disturbance of the colloidal equilibrium of the blood, with consequent fall in leucocytes and platelets, as the main reason for the symptoms observed during anaphylactic and peptone shock. I never understood what point was involved in the idea of the disturbance of the colloidal equilibrium of the blood. There is no question, however, that something remained from this old conception. This refers to the fall in leucocytes and platelets as a characteristic feature of anaphylactic and peptone shock. More detailed studies have subsequently shown that a fall in platelets is one of the most constant indications of a state of sensitization when the antigen is injected, at least in certain species of animals. In 1924, Webb[1] made an extensive study of the leucopenia which occurs in anaphylaxis in the dog, describing enormous accumulation of leucocytes inside of the liver parenchyme, as shown by histological methods. As concerns platelets, Kopeloff and associates[2] and Kinsell and associates[3] have shown that a decrease in platelets is definitely proportionate to the gravity of anaphylactic shock in monkeys and rabbits. At the same time, Kopeloff and Kinsell tried to correlate the rupture of platelets with the liberation of histamine, since rabbits' platelets are very rich in histamine.

The following scheme incorporates the theoretical explanation for their findings:

TABLE 1

INTERRELATIONS BETWEEN PLATELETS' DESTRUCTION AND HISTAMINE RELEASE IN THE RABBIT SUBMITTED TO ANAPHYLACTIC SHOCK*

A. Sensitized animal + antigen → B. Platelet and W.B.C. destruction

Platelet destruction $\xrightarrow{\text{is related to}}$ Changes in blood coagulability

Platelet and W.B.C. destruction $\xrightarrow{\text{liberate}}$ Histamine

Histamine $\xrightarrow{\text{produces}}$ Symptoms and some blood changes

* According to Kinsell *et al.*[3]

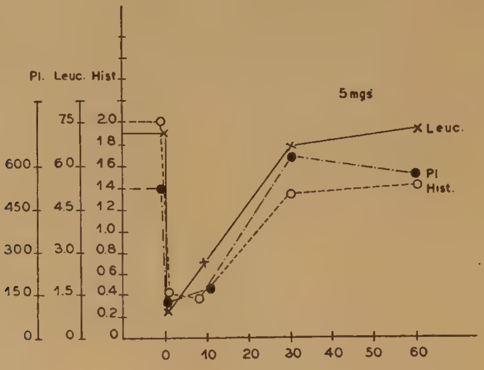
This interesting correlation postulated by Kinsell and Kopeloff, between disintegration of platelets and leucocytes and the liberation of histamine, can only be valid in relation to the rabbit, since, as above stated, rabbits' platelets are a rich source of histamine. But, in the case of the dog, for instance, it is not so easy to explain liberation of histamine as a consequence of an explosive destruction of platelets, since those blood elements are very poor carriers of histamine in this species of animal. Moreover, it has been proved that most of the histamine which appears in the circulating blood during anaphylactic and peptone shock, in the dog, comes out of the liver.

The main attempt undertaken in the last years by myself and co-workers[4–6] has been to correlate the mechanism of liberation of histamine and other metabolites with activation of enzymes of the proteolytic type. It is a known fact that trypsin[4] and such other proteolytic enzymes as papain[5] and ficin[6] liberate histamine and, so far as trypsin is concerned, there is a demonstration that it also liberates heparin,[7] adenosin,[8] and the so-called "slowly reacting substance."[8] There is also ample evidence that, during anaphylactic and peptone shock, activation of proteolytic enzymes takes place,[9–12] and that this activation appears to be correlated with the very mechanism of production of those kinds of shock. This is what might be called the enzymatic point of view, and, according to evidence presented several times, it is not impossible to establish a connection between this enzymatic conception and the other pharmacological and hematological points of view previously mentioned. How it could be done now, with the evidence available, is an important point to be discussed in this paper.

Relationship Between Blood Histamine and White Blood Elements. In 1937, on the basis of a fractionation of heparinized blood from different species of animals, such as the goat, the calf, the dog, the rabbit, and the bullock, as well as from humans, Code[13, 14] concluded that 70 per cent of the blood histamine is bound to the white-cell layer, the so-called "buffy coat." As regards the dog, this connection between histamine and granulocytes, as established by Code in 1937, is probably correct. Also, in humans, it seems quite well established that the main source of histamine in the blood must be found in the leucocytes, although opinions diverge regarding the different lines of white blood cells to which histamine might be linked. On the basis of observation of 5 cases of eosinophilia, Code and Macdonald[15] suggested that eosinophils are the main carriers of histamine in human blood. Randolph and Rackemann,[16] however, could not find any correlation between eosinophilia and the histamine content of the blood. In France, Parrot and Gabe[17] analyzed one case of great eosinophilia, in which a fractionation of the different elements of the blood showed that, although the platelets had the highest concentration of histamine, this base was found in larger amounts in the eosinophils. The neutrophils, according to the French workers, were very poor in histamine.

The most interesting case of the relationship between histamine and white blood elements, however, is presented by the rabbit. The blood of the rabbit is an important source of histamine, each cubic centimeter containing as much as 2 to 5 micrograms of the base. Barsoum and Gaddum[18] showed that the amount of histamine bound to the blood cells was 6 times greater than that of the plasma. Anrep and Barsoum[19] and Code and Ing[20] reported results showing that blood histamine in the rabbit is mainly bound to the leucocytes, since 70 per cent of the total blood histamine extractable from rabbit's blood might be found in the buffy coat. It is, however, a known fact that this buffy coat is usually heavily contaminated with platelets. Therefore, the fact that it is very rich in histamine would not prove that the histamine is mainly bound to leucocytes. Other methods have been used in this connection, and we can say that evidence is now in favor of this histamine's being bound to platelets in the blood of the rabbit. First, we have to mention the experiments of Minard,[21] in which he applied electrophoresis to extract the histamine from several portions of rabbit's blood and showed that histamine is concentrated in the platelets fraction of the blood in amazing quantities. The findings of Minard were extended by Zon, Ceder, and Crigler[22] in experiments in which antiplatelet serum was used to remove platelets from circulation.

The other method used in this connection consisted in following the values for blood histamine after the injection of polysaccharides extracted from certain parasites such as *Echinococcus granulosus* and *Ascaris lumbricoides* and also of glycogen from dog's liver. This method was extensively studied by Graña, Porto, and the author[23, 24] in a series of experiments to which I shall refer in detail. We had observed[23] that, when hydatid fluid (cysts of *Echinococcus granulosus*) was injected into rabbits, it did not produce a fall in blood pressure to contrast with its high toxicity, as observed when dogs are used as animals of experiment. The hematological changes, however, were strikingly similar to those found in rabbits submitted to anaphylactic and peptone shock. Immediately after the injection of the hydatid fluid, leucocytes and platelets fell sharply in the circulating blood and simultaneously the blood histamine showed considerable decrease. When we started studying another helminth, the *Ascaris lumbricoides*, using the extracts prepared according to the technique described by Macheboeuf and Mandoul,[25] we found that extracts which were extremely toxic for guinea pigs and dogs failed to produce any symptom in the rabbit. Yet the same hematologica changes, namely leucopenia, thrombocytopenia, and fall in blood histamine, were olso observed.[24] Fractionation of the hydatid fluid showed that a polysaccharide fraction was mainly responsible for this triad of hematological changes, analogous to those which are typical of anaphylactic and peptone shock. Similarly, fractionation of the *Ascaris* extracts employed has shown that glycogen-like material, which constitutes a substantial part of these extracts, was mainly responsible for the effect upon leucocytes, platelets and histamine when injected intravenously into rabbits.



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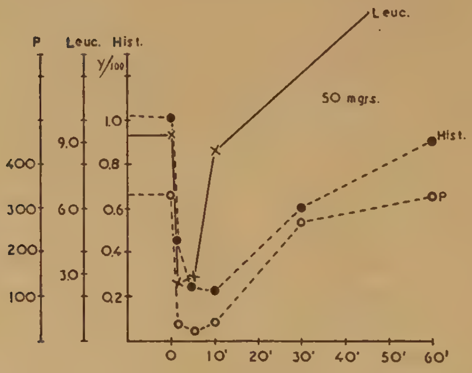

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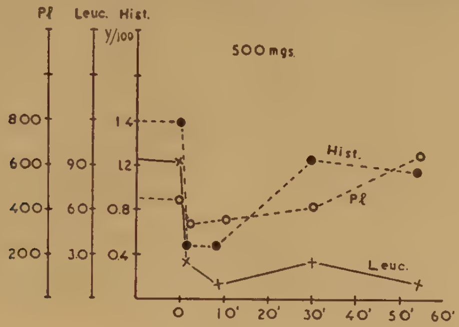
FIGURES 1, 2, 3, and 4. Variations of leucocytes, platelets, and blood histamine in normal rabbits receiving, intravenously (at 0), from 5 mg. to 500 mg. of liver glycogen per kg. of body weight. Note that the curves for platelets and histamine show a fairly good parallelism.

These findings led us to try a purified glycogen, extracted from dog's liver. It was shown that glycogen produces a sharp fall in leucocytes when injected into several species of animals.[26] We found that, at the same time, the platelets fall sharply and, also, that histamine is sharply decreased when very small doses of glycogen are injected into the veins of rabbits. FIGURES 1, 2, 3, and 4 show this effect of doses of glycogen ranging from 5 mg. to 500 mg. per kg. of body-weight. Compared with other substances, such as gelatin and agar, the ability of glycogen to produce drastic changes in the leucocytes and platelets and also in the blood histamine, is strikingly higher.[27] If one analyzes carefully the curves presented in FIGURES 1–4, one can see that, although in some cases the curves for leucocytes, platelets, and histamine run a parallel course, in a few instances the leucocytes rose very sharply after an initial fall, while the curves for platelets and histamine were still depressed. In other cases, the opposite was observed, the leucocytes remaining low (FIGURE 4), while the histamine and platelets went up more swiftly. In all cases examined, it appeared quite evident that the histamine curves ran more parallel to the platelet curve than to the leucocyte curve. This is another excellent indication that histamine is mainly bound to platelets. In the experiments with hydatid fluid and *Ascaris* extracts, a similar parallelism was observed.[23, 24]



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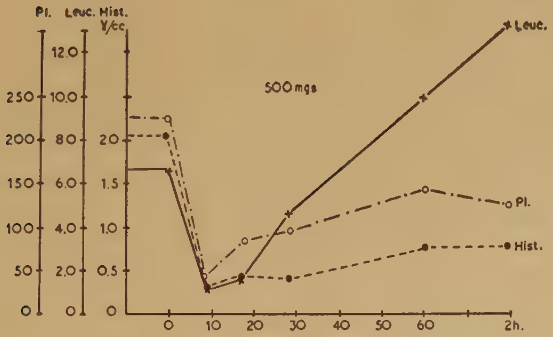

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Segregation of Leucocytes and Platelets in the Small Vessels of the Organs. Another interesting observation in this series of experiments was the fact that, by injecting small amounts of glycogen, we could increase conspicuously the histamine content of an organ, such as the lung. The experiments were done on rabbits in which thoracic aorta and inferior vena cava were clamped just above the diaphragm. In this "thoracic rabbit," if one injects glycogen intravenously, taking pieces of the lung before and after the injection, a considerable increase of the lung histamine, up to 7 times the previous quantity, could be observed (FIGURE 5). I call attention to this possibility of modifying the actual amount of histamine bound to the tissues by simply changing the distribution of blood cells, especially leucocytes and platelets. Under the influence of glycogen, these elements are agglutinated and the agglomerates are captured by the lungs, forming clumps. It is obvious that an accumulation of elements which are very rich in histamine, inside an organ parenchyme, would lead to an increase of the actual amounts of histamine extractable from that organ.

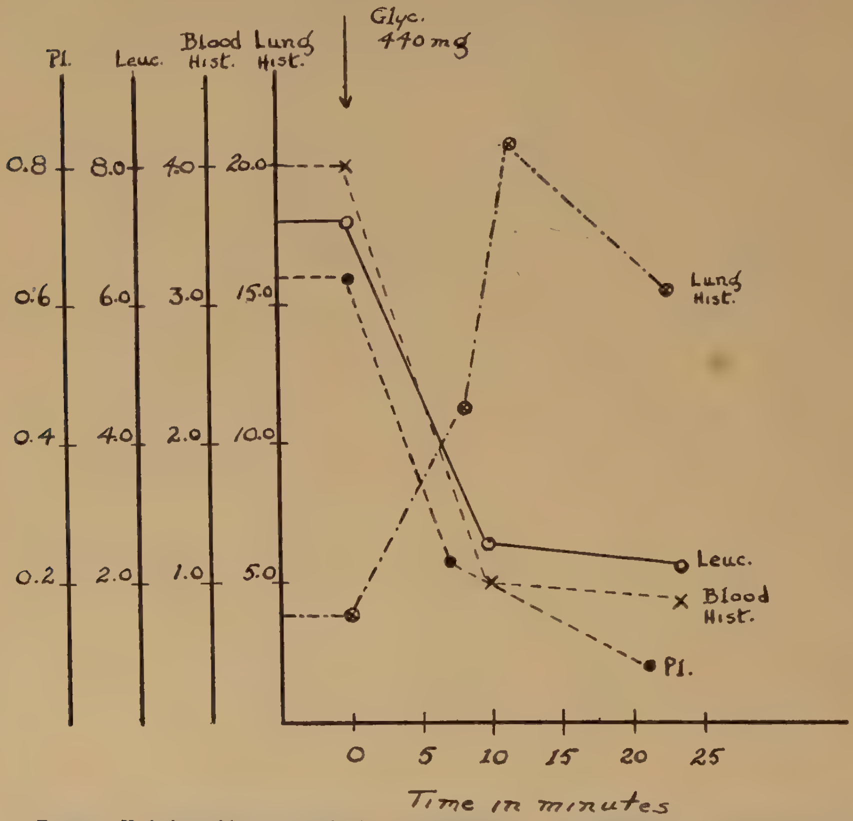

FIGURE 5. Variations of leucocytes, platelets, blood histamine, and lung histamine, after the injection of 440 mg. of liver glycogen per kg. of body weight, in a "thoracic rabbit" (inferior vena cava and thoracic aorta clamped just above the diaphragm).

I wish to emphasize that neither glycogen, hydatid fluid, nor *Ascaris* extracts produce any toxic effect on the rabbit. Therefore, a simple removal of the white blood elements from circulation, in the form of agglomerates or clumps, is entirely incapable of producing any symptom of shock. Those polysaccharides are devoid of the capacity of liberating histamine from the blood cells when added *in vitro* to samples of rabbit's blood.[23, 24] In similar conditions, as we shall see later, the antigen or peptone produces a release of histamine. This can be shown by estimating plasma histamine with and without the addition of antigen and peptone. Another difference can be found in the fact that the aggregates of leucocytes and platelets, as produced by glycogen, remain intact inside of the capillaries of the lung or the liver. This can be shown by using a very simple technique to see the clumps of white blood elements inside of the organs. Since this technique constitutes an important source of information for the role played by leucocytes and platelets in anaphylactic and peptone shock, I shall describe it in detail.

It is evident that simple counting of leucocytes and platelets in the circulating blood will not give very much information on the real fate of those blood elements. By simply counting, we cannot decide whether these blood elements are only segregated or actually destroyed. To decide whether there is or is not destruction of platelets after they are agglutinated, we had to devise a technique to see these elements inside of the organs themselves. After several trials, we could obtain consistent results simply by cutting a small fragment of the organ within a 3.8 per cent solution of sodium citrate. An angle of the organ was dipped into the anticoagulant solution contained in a shallow dish and a small piece cut with sharp scissors beneath the surface of the liquid. The pieces were again cut into smaller fragments, and smears or impressions were made over slides and stained with Giemsa or May-Grünwald. When the pieces were taken from normal organs, a fairly uniform distribution of the platelets could be seen in the smears. If glycogen, for instance, is injected into a "thoracic rabbit," and smears of the lung made as described, enormous aggregates of platelets and leucocytes are seen in the slides. Those aggregates, formed after the injection of glycogen or any of the aforementioned polysaccharides, are stable, contrasting with the explosive destruction of platelets seen after the injection of peptone or the antigen, as will be referred to later.

To finish this subject, I should like to give a few indications of the possible mechanism by which glycogen might work in agglutinating platelets and leucocytes. There is no question that the blood elements become more sticky after the injection of glycogen. This fact has been shown by Essex and Graña,[28] using a transparent chamber adapted to the rabbit's ear. It is also apparent that glycogen does not work simply by its colloidal properties, since other colloids, like gelatine and agar-agar, are extremely less active in removing leucocytes and platelets from circulation than liver glycogen.[27] In this connection, it is interesting to recall some old experiments by Chambers and Grand,[29] in which they found that glycogen displays an extremely potent chemotactic effect upon leucocytes. This observation was confirmed and extended by Delaunay.[30] Although a further investigation is necessary to clarify the mechanism by which such minute amounts of glycogen produce a fall in platelets and leucocytes, it is unquestionable that glycogen might constitute an important tool as a reagent to remove leucocytes and platelets momentarily from circulating blood. This technique was applied, in a few instances, to study the role played by leucocytes and platelets in those kinds of shock, as will be mentioned later on; but it is necessary to use the information obtained by this procedure with caution, since leucocytes and platelets are not removed from the body, but simply segregated in several unspecified structures. Therefore, the animal cannot be compared with a deplatelized or agranulocytic animal for all purposes. Some information, however, could be obtained simply by removing these blood elements from circulating blood.

Anaphylaxis in the Rabbit. The most interesting aspect of the relationship between blood elements and blood histamine can be studied as regards anaphylactic and peptone shock in the rabbit. In 1938, Abell and Schenck[31] observed the behavior of arterioles and capillaries, using the transparent moat chamber in the rabbit ears. After the injection of the antigen into sensitized animals, they observed arteriolar contraction with obliteration of the lumen, increased adherence of leucocytes to the endothelium of the blood vessels, and leucocytic migration through the walls of the capillaries and venules. Particularly interesting was the fact that leucocytes become sticky, adhering to form clumps or emboli and producing, in certain cases, stoppage of the circulation in the small vessels. Endothelial destruction with blood extravasation was seen in the most severe reactions.

That this clumping of leucocytes is of interest in explaining some of the peculiarities of the anaphylactic crisis in the rabbit, particularly the leucopenia, was shown by Dragstedt and associates[32, 33] in experiments of lung perfusion of a normal rabbit with heparinized blood, to which a mixture in optimal proportions of the antigen with the antibody was added. Leucocyte counts were made before and after the addition of the shock-causing mixture. There was a marked reduction in the leucocyte counts, amounting to approximately 50 per cent of the previous values, immediately after the first passage of the blood containing the antigen-antibody mixture. The conclusion to be taken from these experiments is that the lungs, or possibly other organs luxuriantly provided with capillaries and small vessels, may act as filters for the leucocytes and possibly also for the platelets, which become sticky after the injection of the shocking mixture. Since the lung constitutes the organ of shock in the rabbit, we can understand the significance of this plugging of the small vessels of the lung with microthrombi or emboli formed by agglutinated blood elements for the aggravation of the occlusion of the pulmonary artery bed.

There is, however, another aspect of this problem that must be analyzed here. The white cells of the rabbit (leucocytes and platelets) are the main bearers of blood histamine. Rose and Weil[34] and Rose[35] have shown that blood histamine sharply decreases in anaphylactic and peptone shock in the rabbit, and Goetzl and Dragstedt[36] have shown that a similar phenomenon takes place when peptone is injected intravenously in this kind of animal. Part of this decrease of blood histamine can be accounted for by the mechanical removal of white blood cells (leucocytes and platelets) from the circulation. It is conceivable that, if the main carriers of histamine in rabbits' blood, namely leucocytes and platelets, are retained in some fixed structures, such as the capillaries and small vessels of the lung, the total histamine content of the blood must drop in the same way as in the experiments described with glycogen and other polysaccharides.

There is, however, a substantial difference between the experiments performed with glycogen and those in which the antigen or peptone is injected or added *in vitro* to samples of rabbits' blood. The difference consists in the more or less rapid diffusion of the histamine from cells to plasma, as shown first by Katz[37] and confirmed by others.[32, 33, 38] Rabbits sensitized to egg albumin were bled by cardiac puncture and the blood heparinized and submitted *in vitro* to the contact with the antigen. In all instances, the plasma of the blood, which had undergone this *in vitro* "shock," showed marked increase in the amount of histamine, indicating an ability of histamine to diffuse from cells to the surrounding plasma as a consequence of the presence of the antigen.

Dragstedt, Ramirez, and Lawton[32] confirmed these findings of Katz and calculated from *in vitro* experiments that the amount of histamine which could be liberated *in vivo* from rabbits' blood-cells would be about 0.1 to 0.3 mg. per kilogram of body weight. This amount is not enough to produce death in a rabbit when injected intravenously, but we must consider that anaphylactic shock in the rabbit very seldom follows a very severe course and that the histamine is probably liberated from the clumps of leucocytes and platelets formed in the intimacy of the capillaries and small vessels. Therefore, even minimal amounts of histamine might produce considerable pharmacological effects. It is logical, however, to assume that, besides the histamine which is liberated from the white cells, the mechanical plugging of capillaries with microthrombi formed by agglutinated blood elements might constitute an aggravating factor. Evidence for this interpretation was recently afforded,[24] by injecting large doses of glycogen in sensitized rabbits before the injection of the antigen. Leucocytes, platelets, and blood histamine were drastically reduced by the glycogen injection and the animal became refractory to further injections of the antigen. It appears quite clear that the previous injections of glycogen would disperse leucocytes and platelets all over the body, without allocating them to any specified vital structure, although the antigen or peptone would allocate them predominately to the capillaries and small vessels of the lungs, where the main events of anaphylactic shock take place.

At this point, I would like to formulate a set of general statements: (1) the release of histamine is connected with some kind of breakdown of the white blood elements; (2) the clumping of the white blood elements is a constant event during anaphylactic and peptone shock; (3) histamine might constitute a localizing factor for the capture of leucocytes and platelets, which have already begun to clump in the peripheral circulating blood; (4) obstruction of the small vessels and capillaries might depend upon two main factors, one chemical, probably histamine, and the other mechanical, the agglutinated white blood elements. In this explanation, the characteristic pharmacological effect of histamine, *i.e.*, the constriction of the pulmonary artery branches in the rabbit would imprint its main pattern on the anaphylactic reaction by localizing the clumped elements to the capillaries of the organ of shock, the lung.

Anaphylaxis in the Dog. In 1924, Webb[1] made an extensive study of the leucopenia which occurs in sensitized dogs as a consequence of the injection of the antigen. Leucocytes were found in enormous quantities, especially in the liver. More recent studies dealt with the counting not only of leucocytes in the circulating blood but also of platelets.[39, 40, 12] There is no question but that the fall in platelets and leucocytes follows the same trend of the other characteristics of anaphylactic and peptone shock, but a strict parallelism could not be found so far as anaphylactic shock is concerned. It is a known fact that sometimes the platelets and also the leucocytes decrease sharply in circulating blood, after the injection of the antigen, without very much impairment in the blood pressure.[39, 40] This would prove that a fall in leucocytes and platelets is not enough to produce shock. In the case of peptone shock, however, the parallelism between the decrease in platelets in circulating blood and the fall in blood pressure is quite striking, as are the other characteristics of the shock, such as increase in blood histamine and heparin.[12]

There is, however, another point that has been overlooked by previous workers, namely destruction of platelets. Lysis or disintegration of these blood elements cannot be shown simply by counting them in peripheral blood, since they might be retained by some organ structures. Furthermore, disintegration occurs after the injection of glycogen. It is obviously necessary to see whether those clumps formed inside certain organs are or are not destroyed or disintegrated. We have been able to demonstrate this by using the technique, previously described, of making smears of pieces of certain organs for microscopic observation (FIGURE 6, A). In the intact dog, if one injects the antigen or peptone and takes a fragment of the liver immediately after the injection, one can see (FIGURE 6, B) enormous aggregates of platelets and also of leucocytes, scattered all over the slides. If the fragment is taken a few minutes later, a gigantic destruction of these clumps begins to occur, sometimes taking the course of an explosive lysis or disintegration of platelets. In their places, we can see, after a short while (FIGURE 6, C), only ghosts of the previous clumps, or a dust-like material, staining very well by Giemsa or May-Grünwald and unquestionably identifiable with remains or debris of platelets. By this direct method, we can therefore show that platelets are really disintegrated during shock.

As we saw in the case of the rabbit, it has been possible to establish a certain relationship between breakdown of platelets and the release of histamine that takes place when the antigen or peptone is put into contact with samples of rabbits' blood. Conditions in the dog are entirely different, since the histamine which appears in circulating blood during shock comes mainly from the liver. Is it possible, however, to establish a similar relationship, even in the case of the dog?

The participation of the liver in the production of anaphylactic and peptone shock has been suspected since early times. Manwaring[41] was unable

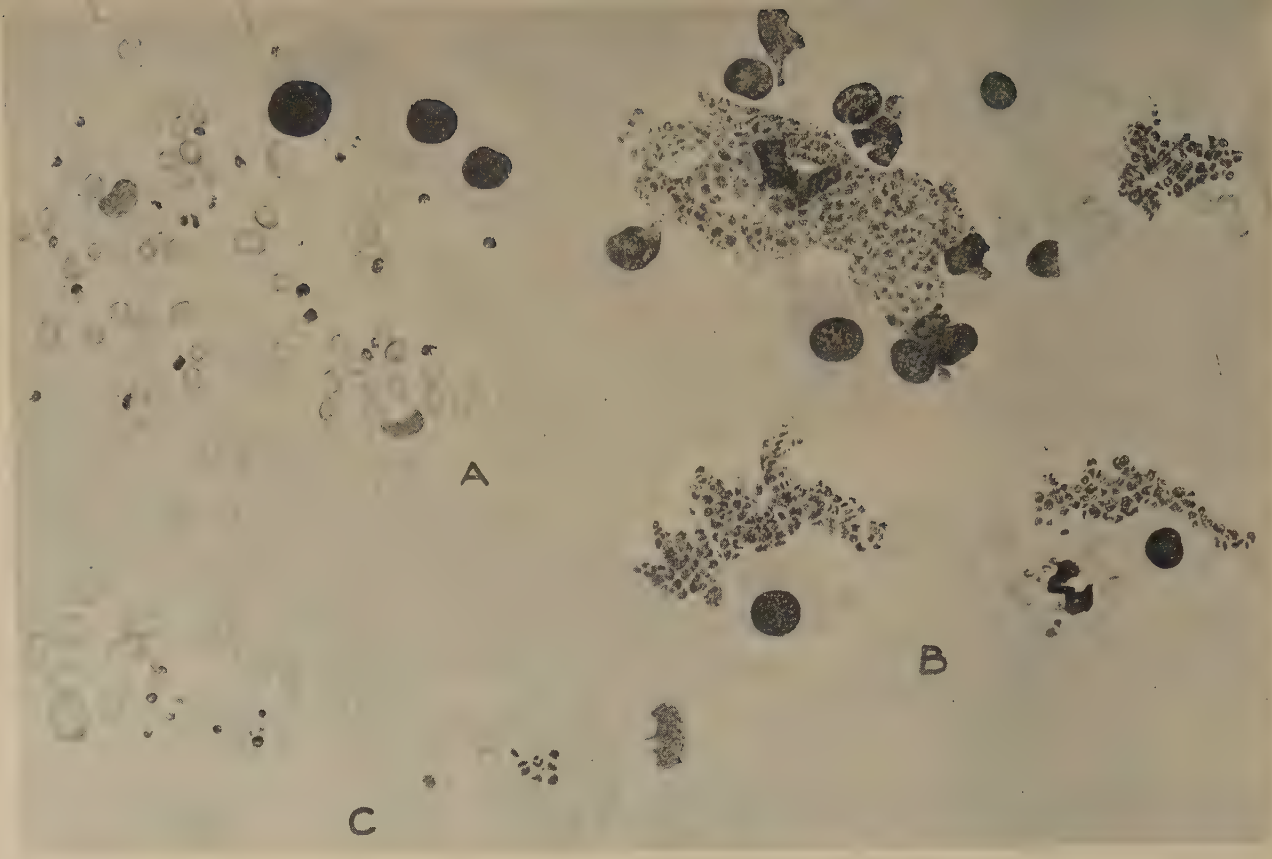

FIGURE 6

A. Smear of a piece of normal dog liver, taken directly into a 3.8% solution of sodium citrate. Stained by May-Grünwald. Note the homogeneous distribution of platelets among red cells (450× enlargement).

B. Impression over slide of a piece of the liver of a dog submitted to shock by injection of the antigen from *Ascaris lumbricoides*. Enormous clumps of platelets are seen in the slides, made with a fragment of liver taken before 2 minutes after the injection of the shocking agent (450× enlargement).

C. Impression over slide of a piece of the liver of the same animal, 10 minutes after the injection of the *Ascaris* antigen. The clumps previously seen, are entirely disintegrated and in their place only remains of platelets or ghosts of clumps can be seen (450× enlargement).

to elicit anaphylactic shock after ligation of the aorta and inferior vena cava just above the diaphragm. Also, the injection of the antigen in a sensitized dog with an Eck fistula does not produce shock, as shown by Voegtlin and Bernheim[42] and Denecke.[43] The extreme stagnation of blood in the portal region was already described by Richet,[44] and shown by Weil[45] to be quantitatively enough to explain fall in blood pressure and death of the animal. More recently, Ojers, Holmes, and Dragstedt[36] showed that most of the histamine appearing in the circulating blood comes out of the liver, since histamine estimations of pieces of the liver taken before and after the injection show considerable decreases following the development of the shock. A similar discharge of histamine from dogs' liver in peptone shock was described by Holmes, Ojers, and Dragstedt.[47] That heparin is also liberated from the liver has been shown repeatedly.

In 1943, Graña and the author[48] decided to show by perfusion experiments the hepatic origin of this histamine that appears in the blood of the dog during anaphylactic shock. We started using Tyrode solution as vehicle for the perfusion. To our great astonishement, not a single μg. of histamine or heparin appeared in the perfusates after the injection of the antigen. Using peptone, we could detect a few micrograms of histamine (not more than 10 or 12), contrasting with the milligrams which are released in the intact animal, but no heparin could be detected.[12] Later on,[49] we decided to experiment by using citrated blood contained in paraffined receptacles. Under these conditions, some histamine and heparin were released, but not as much as can be released *in vivo*. Finally, in collaboration with Scroggie, Fidlar, and Jaques[7] we decided to exclude the anticoagulant from our experiments of liver perfusion, using blood kept in its natural conditions by the use of flasks covered with silicones. As shown by Jaques *et al.*,[50] the blood is kept unclotted for at least 2 hours, and even platelets are well preserved during the first half-hour after collecting the blood. By adding peptone to that blood, and pumping it through the liver, we have been able to detect enormous amounts of histamine and heparin, which were liberated in quite an explosive way during the first minutes of perfusion. As much as 8.5 mg. of histamine and more than 50 mg. of heparin could be liberated in one of the experiments of liver perfusion with silicone blood plus peptone.

These experiments provided the final proof that the blood contains a factor or factors which are necessary for the discharge of histamine and heparin from the liver of the dog during anaphylactic and peptone shock. Since the anticoagulant seems to constitute a disturbing factor, and the best results were obtained when the blood was kept as near as possible in its natural condition, we have to conclude that the most labile part of the blood, namely leucocytes and platelets, are probably involved in the production of this discharge of histamine and heparin. What was the fate of these blood elements after perfusion of the liver with citrated or silicone blood? In all those experiments, leucocytes and also platelets were counted before and after the passage of blood plus antigen or peptone through the isolated organ. Immediately after the injection of the antigen or peptone, leucocytes and platelets were instantaneously picked up and were found to be forming aggregates inside the organ parenchyme. If citrated blood is used for the perfusion, those clumps of leucocytes and platelets are found well protected and intact at the end of the perfusion. Also, the amounts of histamine and heparin liberated under these conditions were rather small. If, however, silicone blood is used for the perfusion, leucocytes and platelets are likewise picked up by the organ, but the clumps formed are found to be completely disintegrated at the end of the experiment and enormous amounts of histamine and heparin can be detected in the perfusates. This parallelism between destruction of white blood elements and the discharge of histamine and heparin from the liver cells, which is so striking in the experiments with the isolated liver of the dog, can also be found *in vivo*, if fragments of liver are taken before and after the injection of the shocking agent.[49]

Other evidence pointing in the same direction was given by experiments in which liver glycogen was injected into the intact dog before the *Ascaris* extract. As previously mentioned, extracts prepared from *Ascaris lumbricoides*, according to the technique of Macheboeuf and Mandoul,[25] produce a profound shock in the dog that cannot be distinguished from anaphylactic shock.[48, 49, 51] Enormous amounts of histamine are liberated from the liver and can be estimated in the circulating blood. Simultaneously, a profound fall in platelets and leucocytes takes place in the circulating blood and, if pieces of the liver are taken for microscopic observation, enormous aggregates of platelets and leucocytes can be found in the piece taken immediately after the injection (FIGURE 6). If another piece is taken a few minutes after the injection, a considerable destruction of the formed clumps can be observed, amounting to a real "explosion" of platelets. Leucocytes also are shown to be heavily damaged. If, before the injection of the *Ascaris* extract, we inject a large dose of glycogen, 3 to 8 gm. in 10 to 20 cc. of saline, platelets disappear almost completely from the circulating blood and no shock occurs. If then, the *Ascaris* extract is injected, the effect is somewhat modified, as concerns the fall in blood pressure, since the shock is definitely mitigated.[48] What is striking, however, is the almost complete inhibition of the increase in histamine and heparin in the circulating blood. The interpretation given to this effect of a previous injection of glycogen was that a dispersion of the white blood elements would prevent any serious accumulation of the same inside the liver parenchyme, thus bringing about a situation unfavorable to the discharge of histamine and heparin.

We have evidence, therefore, that in the dog the discharge of histamine and heparin from the liver parenchyme depends upon a previous breakdown of the white blood elements: (1) the blood is important for the discharge, and the natural condition of the blood must be preserved in order to have a maximal liberation of histamine and heparin from the isolated liver; (2) disintegration of platelets and leucocytes is somewhat correlated with the intensity of the shock and also with the discharge of histamine and heparin from the liver; (3) a previous dispersion of the white blood elements, by the injection of high doses of glycogen, partially desensitizes the animal to shock and prevents liberation of histamine and heparin from the liver of the dog, kept *in situ*. To this indirect evidence, I might add the fact that the liver of the sensitized dog, when perfused with citrated or heparinized blood, picks up sharply the white blood elements, which are then retained inside of the organ, forming enormous aggregates or clumps. It is logical to assume that these microthrombi might help in occluding the small vessels of the liver, already partially occluded by the action of minute amounts of histamine.

On the basis of these facts, one might draw the following scheme for the mechanism of anaphylactic and peptone shock in the dog: (1) immediately after the injection of the antigen or peptone, there occurs an initial constriction of the hepatic vessels, effective in transforming the capillaries of the liver into a filter for the agglutinated blood elements; (2) the leucocytes and platelets agglutinated in contact with the antigen will form microthrombi in the small vessels and capillaries of the liver; (3) after the latent period, the platelets will "explode," this being followed by the liberation of histamine and heparin.

Activation of Proteolytic Enzymes during Anaphylactic and Peptone Shock. By which mechanism are products derived from platelets or leucocytes concerned with the liberation of histamine and heparin from dog's liver? It is possible that leucocytes and platelets might contain an enzyme of the proteolytic type, the release of which, during shock, might bring about the liberation of those metabolites. We have now, however, several indications that the protease system of the blood itself might constitute the missing link in the chain of events leading to the release. This plasma protease has been described in the literature under the names of "plasma trypsin,"[52] "plasma tryptase,"[53] "lytic factor,"[54] "plasmin,"[55] and, more recently, "fibrinolysin."[56] The difficulty in showing its activation during peptone and anaphylactic shock resided in the fact that the blood becomes incoagulable after shock, due to the discharge of heparin. Therefore, if no clot is formed, it is obvious that the fibrinolytic effect could not possibly be observed.

To overcome this difficulty, we have used the protamine test, as described by Jaques and Waters.[57] After addition of a suitable amount of protamine, the blood clots. If the samples are incubated at 39°, a more or less rapid redissolution of the coagulum can easily be observed, especially in the tubes containing the largest amounts of protamine. It appears quite clear that protamine disposes not only of the heparin released, but probably also of the inhibitor present by fixing it up as soon as its connection with the enzyme had been broken down during shock. Using this fibrinolytic protamine test,[11, 12] we have been able to correlate the intensity of the fibrinolytic effect to the degree of shock: fibrinolysis is maximal after a first injection of the shocking agent, moderate after the second injection of peptone, and null after a second injection of the antigen or a third injection of the peptone. All the other symptoms of the shock, such as fall in blood pressure and liberation of histamine and heparin from the liver, follow the same trend. This led us to the conclusion that the fibrinolytic effect is connected with the mechanism of production of those kinds of shock, and that desensitization might depend either on an exhaustion of the mechanism leading to fibrinolysis or on the discharge of any antifibrinolytic agent, which would block the first wave of fibrinolysis. Probably both mechanisms might be operative in normal conditions.

That this antifibrinolytic agent seems to come from the liver was suggested by experiments performed in the so-called "anterior animal" or "thoracic dog." This kind of preparation, already used by Nolf,[9, 10] can be set up simply by clamping thoracic aorta and inferior vena cava, just above the diaphragm. In that case, the fibrinolytic effect is very strong and much more pronounced and lasting than that observed in the intact dog, when peptone is injected. After the injection of peptone in the thoracic animal, we can observe a strong fibrinolytic effect until the end of the experiment, and no heparin appears in the samples. Therefore, we can be sure that all the samples taken from a thoracic animal, after the injection of peptone, will fibrinolyse. In this preparation, there is no recovery after the development of the fibrinolytic activity. Using this preparation, we have been able to demonstrate that a previous injection of heparin can prevent the activation of the fibrinolytic enzyme. In the protamine test, heparin does not prevent the effect of the fully activated enzyme, since, when the blood is collected in a syringe containing an excess of heparin and then distributed over the tubes of the protamine series, it will undergo fibrinolysis (as will the controls) without heparin. When heparin is injected before the peptone, however, it will sometimes entirely block the development of the fibrinolytic phenomenon in the thoracic preparation (FIGURES 7 and 8).[58]

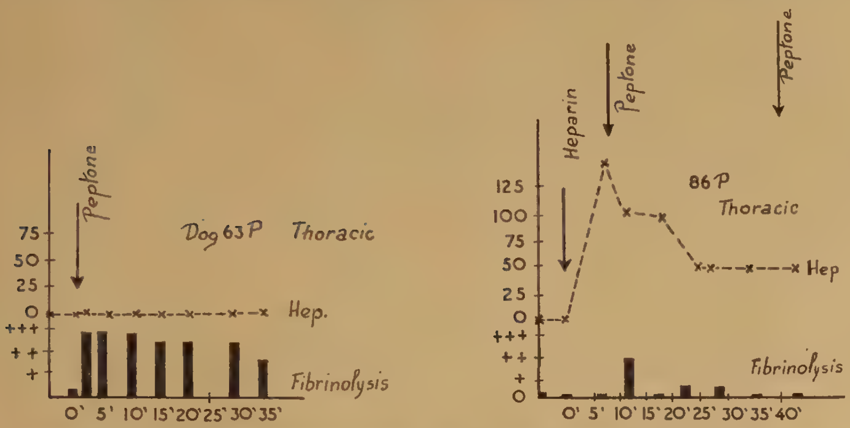

FIGURE 7. *Left:* Fibrinolytic effect developed in a "thoracic" dog, after the injection of 150 mg. of peptone (Bacto-peptone) per kg. of body weight. *Right:* Before the injection of peptone in a "thoracic" dog, 6 mg. of heparin per kg. of body weight were injected. Fibrinolysis was considerably reduced. In both experiments, the heparin curves indicate the amounts of protamine per 0.5 ml. of blood, that brings coagulation time to a minimum (+++ means fibrinolysis in less than 1 hour; ++, in less than 2 hours and +, in 3 hours or more).

The last point I wish to emphasize is concerned with the fate of platelets in this thoracic preparation. Immediately after the injection of peptone, the platelets and leucocytes disappear from circulation and are found forming aggregates in the intimacy of the lung parenchyme. In order to see these clumps, pieces of the lung were taken directly into a 3.8 per cent solution of sodium citrate, as described before. Immediately after the injection of peptone, we can detect a great number of clumps scattered all over the slides. These clumps are formed by a great number of platelets surrounding several leucocytes. If five or ten minutes are then allowed to elapse and another piece of the lung taken in citrate, the smears will show all those clumps entirely disintegrated and destroyed. In their place, one can see only debris of platelets, formed by small granulations well stained by Giemsa and May-Grünwald. Only exceptionally, one can detect small but heavily damaged clumps of leucocytes and platelets. Also, the fibrinolytic activity of the blood is fully developed in this case.

Now, if heparin is injected before peptone, the platelets will clump as before, but the clumps can be seen to be well formed and intact long after the injection of peptone. I think that this effect of heparin, protecting the clumps of platelets against destruction and, at the same time, preventing activation of the fibrinolytic enzyme of the blood, constitutes good evidence that products derived from platelets might be concerned with the activation of this fibrinolytic enzyme of the blood during anaphylactic and peptone shock.[58]

It is a known fact that this plasma protease is normally present in the blood in an inactive form[55, 59] and that, at the same time, there is an inhibitor preventing it from becomine apparent.[52] Since this enzyme appears to be spontaneously activated under certain morbid conditions, as in traumatic shock,[60] it is obvious that there must be an endogenous activator that might also be mobilized in cases of anaphylactic and peptone shock. As previously shown, there are indications that platelets might provide for this activator. As concerns the mechanism of activation of this enzyme, it might depend not only on a decrease of the inhibitor, allowing the spontaneous activation of the enzyme to take place, but also on the actual activation of the enzyme precursor (tryptogen,[53] plasminogen,[55] or pro-fibrinolysin,[56] according to the name given to the active enzyme) by a kinase, since it has been shown[59] that activation can take place without removal of the inhibitor. This last possibility seems to be the case in anaphylactic and peptone shock, since the samples of blood taken after shock, showing strong fibrinolytic effect when added immediately to the protamine series, will return to normal, after a few hours at room temperature, if no protamine is added at the beginning. This experiment shows that the enzyme recom-

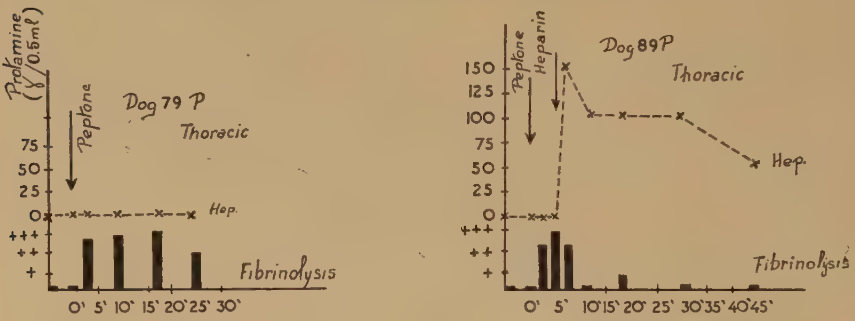

FIGURE 8. *Left:* Fibrinolytic effect in a "thoracic" dog after the injection of 150 mg. of peptone (Bacto-peptone) per kg. of body weight. *Right:* Before the injection of peptone in a "thoracic" dog, 6 mg. of heparin per kg. were injected; activation of the fibrinolytic power was stopped in a very early stage. (Signs and methods the same as in FIGURE 7.)

bines with the inhibitor still present in excess in the blood samples and that, if the blood is kept unclotted (without addition of protamine) by the presence of the released heparin, no fibrinogenolysis occurs, since, if protamine is added a few hours later, the samples will clot but no lysis will occur.

Probable Chain of Events Leading to Histamine Release. Recently,[49] we have drawn up a scheme which attempts to correlate all the facts here described in a chain of enzymatic and pharmacological reactions (FIGURES 9 and 10). Under the present circumstances, this scheme does not pretend to be more than a simple working hypothesis, the fate of which will depend upon future investigations. Up to now, however, all the facts described in the literature appear to fit quite well into its lines. It is quite probable

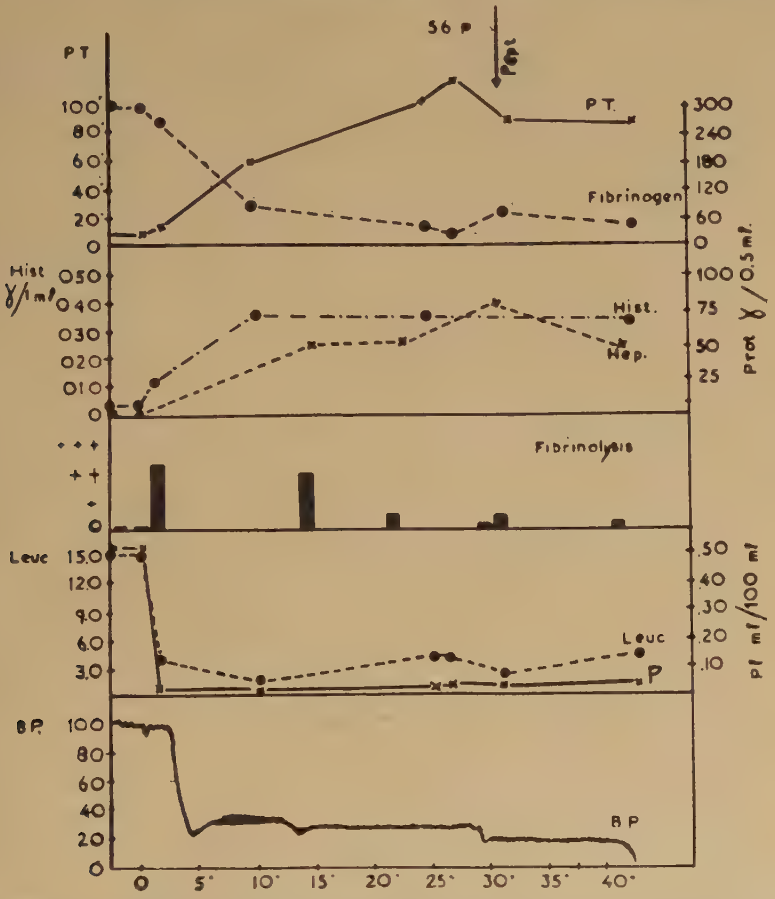

FIGURE 9. Anaphylactic shock in a dog sensitized to horse serum. At 0, 1 ml. of horse serum per kg. of body weight was injected intravenously. The stratified graph shows all the variables of the shock, from down up: fall in blood pressure (B.P.); fall in leucocytes (Leuc.) and platelets (P); the fibrinolytic effect as shown by the "fibrinolytic protamine test," increase in histamine and heparin in the peripheral blood; decrease in the "estimated" fibrinogen and increase in prothrombin time (P.T.). The two last changes are mainly due to increase in blood heparin.

that a similar chain of events might work in the case of the rabbit, with the difference that the main organ of shock in the rabbit is the lung, but, since platelets are a rich source for histamine in this species of animal, it is obvious that a simple breakdown of platelets might liberate enough histamine to explain shock in the rabbit. In the guinea pig, conditions seem to be even simpler, since the direct contact of the antigen with the cells of the organ of shock (the lung) is enough to produce the discharge of histamine, as shown in the classical experiments of Bartosch, Feldberg, and Nagel[61] and in those later on described by Ungar and Parrot[62] and Schild[63] with isolated pieces of several organs. It seems, however, that here an enzymatic mechanism also appears to be operative, since Ungar[64] quite recently showed activation of a fibrinolytic enzyme when organs of the guinea pig are put into contact with the antigen or with peptone.

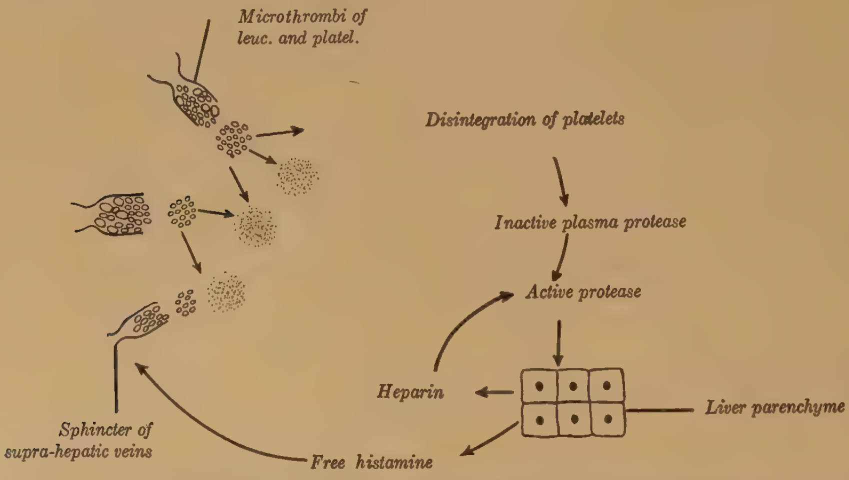
Microthrombi of leuc. and platel.
Disintegration of platelets
Inactive plasma protease
Active protease
Heparin
Liver parenchyme
Sphincter of supra-hepatic veins
Free histamine

FIGURE 10. Probable chain of events leading to the discharge of histamine and heparin from dog's liver, in anaphylactic and peptone shock (according to Rocha e Silva, Porto, and Andrade[49]).

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Discussion

E. Fidlar (*Department of Physiology, University of Toronto, Toronto, Canada*): If platelet disintegration yields an activator for plasma trypsin, why is the latter not active in those cases of experimental purpura where platelets may suddenly be destroyed? Perhaps the trypsin is active; I do not know.

M. Rocha e Silva: This is a very interesting question. It opens up the problem of the relationship between the mechanism of purpura and histamine release in anaphylactic and peptone shock. It is a known fact that thrombocytopenic purpura is a common symptom of hypersensitivity, as, for instance, in cases of drug allergy. The mechanism of production of the hemorrhagic spot is still unknown, but some time ago, in collaboration with Dr. Martins, we studied the mechanism of production of purpura by injection of antiplatelet serum. There is no question but that, under the influence of A.P.S. applied locally to the skin of the dog, rabbit, or rat, platelets clump, forming microthrombi or emboli which disintegrate just before the hemorrhage sets in. Therefore, we might assume that the cause for the hemorrhage in thrombocytopenic purpura is not the absence of platelets, as generally said, but that products of disintegration of platelets liberate or activate a hemorrhagic agent that might be a proteolytic enzyme of the trypsin type, since trypsin itself, always used as a model enzyme to minimize many of the anaphylactic reactions, is strongly hemorrhagic. Moreover, in the preparation described as the "thoracic dog," we have found that injection of a potent antiplatelet serum brings about a slight but detectable activation of the fibrinolytic power of the blood, in a way parallel to the disintegration of the clumps of platelets which form inside of the lung parenchyme.

J. ALEXANDER (*New York, N. Y.*): It is not clear to me as to just how histamine appears and how it acts to cause sudden contraction of certain non-striated muscles.

Since Dr. Rocha e Silva reports that he has observed the clumping of blood platelets and leucocytes in the dog (microthrombi), I trust that the notion of a possible connection between even nonpersistent flocculates and the development of clinical symptoms can be discussed. The experiments of Dr. Auguste Lumière (Le Probleme de l'Anaphylaxie, G. Doin, Paris, 1924) may be recalled in this connection, even though I have been informed that they led to what is now called "anaphylactoid" shock (due to arteriole compression following capillary blockage) rather than to true anaphylactic shock (due to contraction of arteriole musculature).

Lumière found that freshly precipitated barium sulfate, a chemically inert substance, caused no symptoms when intravenously injected into dogs. However, as the precipitate was aged and its particles, as observed microscopically, grew progressively larger, symptoms of increasing severity appeared, leading to violent shock and death, with aggregates of sufficient size. Since the capillary systems of various organs in various animals differ greatly in their vulnerability to blockage by barium sulfate, the clinical consequences vary with the animal used.

I therefore ask: Can flocculates, even if not persistent and not large or firm enough to block capillaries, nevertheless interfere temporarily and locally with supply and elimination, so that cells or other constituents of affected organs or tissues will face changed milieu conditions and react abnormally, one consequence being the release of histamine? Slight interference with blood circulation in the brain may have wide nervous and muscular consequences.

M. L. TAINTER (*Sterling-Winthrop Research Institute, Rensselaer, N. Y.*): The phenomena referred to may be produced by intravenous injection of substances that agglutinate red cells by chemical, physical, or immunological mechanisms. The agglutinated masses block capillaries, especially in the lung bed. These changes have been extensively studied by Hanzlik and Karsner, under the name of "anaphylactoid phenomena." They grossly resemble anaphylactic shock. The mechanism appears to depend on a mechanical effect of compression of the smaller bronchioles by distension of adjacent capillaries.

M. Rocha e Silva: The experiments of Lumière are typical of the kind of explanation that might be classified under the general name of physical theories. They are now well buried under the mass of chemical and pharmacological data presented in the literature since then. I think that Dr. Alexander overemphasized the importance I wished to attach to the mechanical clumping of leucocytes and platelets with the consequent formation of microthrombi. I took for granted that the most important part of the phenomenon should be attributed to disintegration of white blood elements. Simply blockading the circulation of the liver leads to questionable, if any, release of histamine and heparin. It would, by no means, be enough to explain the explosive discharge that sometimes occurs in a few minutes, as shown in the experiments of perfusion of the isolated liver of the dog with peptone. I tried to make clear that products derived from platelets, and in smaller scale from leucocytes, might initiate a chain of enzymatic reactions, leading to the release of metabolites. It is true that I described[48] an instance in the dog, that I tentatively called "mechanical shock," in which the animal appeared to die by a mechanical stoppage of the circulation of the liver without very much discharge of histamine and heparin into the circulating blood. I should not like, however, to give too much emphasis to the "mechanical" nature of this kind of shock.

C. F. Code (*The Mayo Clinic, Rochester, Minnesota*): Since MacIntosh and Paton have recently shown that simple substances like the tripanocidal diamidines, and the even simpler straight-chain diamines, diamidines, and diguanidines, release histamine from tissues, should not the possibility be considered that a simple substance or a group of simple substances similar in action to these compounds may be the chemical mediator for the release of histamine in anaphylactic and peptone shock, rather than the chain of enzymatic reactions described by Dr. Rocha e Silva?

M. Rocha e Silva: The question raised by Dr. Code implies that I am trying to complicate unnecessarily the picture of anaphylactic and peptone shock by bringing activation of a proteolytic enzyme into the picture to account for the liberation of histamine, heparin, and other metabolites. The reasons for bringing this in are presented in numerous publications from my laboratory, and they have been discussed only partially here. There is no question that activation of proteolytic enzymes takes place during anaphylactic and peptone shock and that it might explain a great portion of the symptoms observed, especially the hemorrhagic phenomena (in the intestinal tract of the dog and those that are characteristic of the Arthus' reaction in the rabbit), and necrosis, since histamine is unable to explain these effects. Proteolytic enzymes by themselves, as it was shown for pancreatic trypsin, display a number of pharmacological properties which are closely akin to the symptoms of anaphylactic and peptone shock. Besides that, the fall in platelets and leucocytes, with their consequent disintegration, are also events that happen to occur in those kinds of shock. The discharges of metabolites such as histamine, heparin, adenosine, slowly reacting substance, *etc.*, are epiphenomena occurring after a latent period of one to two minutes.

We might assume that each of the phenomena mentioned are entirely independent entities, or it might also be assumed that they are closely interconnected. In the last case, we have to find out how and why are they interdependent. It is probably simpler to adopt the first attitude and just limit ourselves to a description of all the symptoms of anaphylaxis, making a long catalogue of all data obtained. The second attitude, however, is likely to stimulate research on a more rational basis.

The possibility of the compounds studied by MacIntosh and Paton being the mediators in the production of anaphylactic and peptone shock appears very remote to me. It would be necessary to find out first the mechanism by which these compounds liberate histamine. During the summer of 1947, MacIntosh and Paton had finished the preliminary stage in the study of the release of histamine, and possibly also of heparin, by these compounds. Many questions are still pending further experiment, but it might well be that these compounds release histamine by a mechanism similar to the one shown by peptone, as described in this paper. Another important step would be to identify any of these compounds in the blood or tissues of animals during anaphylactic and peptone shock. In the present status of our knowledge, it seems to me a difficult task to introduce these simple compounds as the mediators for the release of histamine and heparin (and other metabolites) in dog's anaphylaxis.

Discussion of the Foregoing Papers

B. Rose (*University Clinic, Royal Victoria Hospital, Montreal, Canada*): The papers presented thus far have outlined very clearly the various steps in the development of the histamine theory of anaphylaxis and its subsequent application to our understanding of the mechanism whereby shock is produced. It seems curious, however, as pointed out by Sir Henry Dale, that no definite physiological role has yet been ascribed to histamine. Many studies have been made on the blood histamine, and the majority of these determinations have been confined to whole blood. As is well known, the active or free histamine is found only in the plasma.

In this connection, it might be of interest to point out that an increase or decrease in the total blood histamine does not appear to be of great significance *per se.* For example, in the rabbit, it has been demonstrated during anaphylactic shock that a decrease in the total blood histamine occurs, and that this may or may not be associated with a transient release of histamine from cells into the plasma at the very beginning of shock. There can be no doubt that the primary and important change is that small increase in the plasma histamine. This is supported by the observation that an injection of glycogen into the rabbit also causes a drastic reduction in total blood histamine but does not produce shock. Addition of glycogen to the blood of the rabbit *in vitro*, moreover, will not release histamine from the cells.

Again, marked variations in the blood histamine of patients can be shown under certain circumstances. For example, as originally shown by Code, patients with leukemia may have an enormously increased blood histamine (to some 500 to 700 times normal). In these patients, an analysis of the histamine in the blood again shows that the vast majority is bound to some cell constituent. Nevertheless, there may also be a considerable, continuous increase in the plasma histamine. Such patients are neither allergic nor in shock. It would seem, therefore, that in so far as man is concerned one other factor is essential, namely: there must be a hypersensitive target organ. Evidence for this has been presented by previous workers and, in particular, by the recent work of Curry.

It will be recalled that he has demonstrated a reduction in the vital capacity of asthmatic patients following the intravenous or subcutaneous administration of histamine. Such changes do not occur in the nonallergic individual. It is of even greater interest to note that, according to his findings, these patients are even more sensitive to a preparation such as mecholyl than they are to histamine, and that, whereas the effects of histamine can be inhibited by the previous administration of antihistamine compounds, no effect on the action of mecholyl can be induced, as might be expected. To the best of my knowledge, a similar condition does not appear to exist in the sensitized animal, such as the guinea pig; that is, this species is not any more sensitive to histamine in the sensitized condition than it is in its nonsensitized, normal state.

While these points are in no sense a refutation of the histamine theory of anaphylaxis, they are presented as evidence that there seem to be other mechanisms besides the release of histamine in anaphylaxis, particularly with regard to allergic phenomena in man, as pointed out by Professor Dragstedt.

THE DETOXIFICATION OF HISTAMINE

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When considering the mode of removal of histamine from tissues, one might, with considerable justification, substitute the term "inactivation" for the term "detoxification." If further evidence is forthcoming to substantiate the present suggestive evidence that histamine fulfills various physiological roles, then the term "inactivation," applied to what would then be a normal functioning metabolite, would be less open to objection than the term "detoxification." Indeed, as applied to other states, where the quantity of free histamine in the tissues is unusually large, the connotation of the term "inactivation" is less open to objection than the rather popular term "detoxification."

It has been demonstrated many times that, following the parenteral injection of histamine into an animal such as the dog, rabbit, or rat, there is a rather rapid removal of the histamine from the circulating blood and, provided that the dose administered is not too large, the symptoms of histamine shock are ameliorated and finally disappear. Similarly, experiments have been carried out in which various isolated tissues or organs have been perfused with blood containing added histamine. Thus, Best and McHenry[1] and Steggerda, Essex, and Mann[2] showed, in perfusion experiments, that the dog's kidney was the most effective in the destruction of histamine. The liver was less effective, and, in perfusions of the small intestine or of the hind limb, little inactivation occurred. It is with the mechanisms or possible mechanisms of this inactivation of histamine by the tissues that we shall now deal.

It may be that histamine combines, by a simple chemical linkage, with the proteins of cell structures. Thus, it is removed from its sphere of action and is thereby rendered inactive, at least temporarily. This is suggested in many experimental findings and is discussed by some authors.[3, 4, 5] One might also refer to the report of Anrep and his associates[6] that, when histamine is added to a sample of blood, it is, in part, quickly taken up by the red cells and is thereby rendered physiologically inactive. If such blood is extracted for its histamine content, the added histamine may be recovered. We shall return to this aspect of histamine inactivation later.

Inactivation may also occur by conjugation, as shown by the presence in urine of an inactive form of histamine, readily activated by hydrolysis. Inactivation may also occur in tissues, as the result of the activity of the enzyme, histaminase, or, as some would prefer to call it, diamine oxidase.

From time to time, histamine has been detected, usually in small amounts, in the urine of man and other animals. The most thorough study of the presence of histamine in urine has been made by Anrep and his coworkers. They[7] reported in 1944 that, if urine was hydrolyzed with hydrochloric acid, while it frequently showed no histamine activity before hydrolysis, after hydrolysis there was often unmistakable evidence of histamine. They showed that the histamine is excreted in a conjugated and inactive form, which is freely diffusible and from which the active base can be liberated by hydrolysis, either by acid or by alkali. This increase in the histamine-like activity of urine after hydrolysis might have been due either to the destruction of some antagonizing substances interfering with the contraction of the guinea-pig ileum, used in the test, or to a release of the active principle from a conjugated or inactive form. They excluded the first possibility by showing that, when histamine is added even in traces to the nonhydrolyzed extracts, it can be estimated quantitatively on any of the usual test objects. Conjugated histamine, like the free base, is soluble in water and in alcohol and is readily adsorbed from urine by charcoal (B.D.H. decolorizing charcoal), from which it can be released by repeated elution with acidulated alcohol (0.3 N HCl in 95 per cent alcohol). In testing the action of histaminase, they found that the enzyme is entirely inactive in the presence of urine and that this inhibitory influence is retained by the extracts of urine even after acid hydrolysis. The inhibition becomes negligible on dilution. Even so, histaminase does not act on conjugated histamine.

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There was no appreciable difference between the urines of a large number of different species of herbivora examined. The extreme variation was 0.02–0.2 γ/cc. urine, calculated as histamine acid phosphate. There was no marked variation in urine collected from typical carnivora, but the histamine equivalent of all urines was high, 10 to 40 γ/cc.

Animals on a varied diet gave considerable variations in the values of urine histamine. Thus, the white rat showed variations between 0.1 and 10.2 γ/cc. and the dog varied between 0.2 and 45.0 γ/cc. of urine. There appeared to be no relationship between the histamine equivalents of blood and urine, but it appears that the excretion of histamine into the urine depends upon the nature of the diet. In herbivora, histamine is chiefly in the free form, while in carnivora the histamine is completely conjugated. In the urine of the rat and of man, both forms are found.

When histamine was injected subcutaneously into dogs, there was no change in the amount of conjugated histamine in the urine and there appeared only a slight amount of free histamine, which is not usually present. The longer the period of anuria, following the injection of histamine, the smaller was the excretion of histamine. In any case, the bulk of the histamine was destroyed within the body. Histamine is present in meat in the free form, and the increase in histamine after the feeding of meat is partly due to the preformed histamine in the meat, but apparently not wholly so. The feeding of large amounts of histamine (up to $\frac{1}{2}$ g.) to dogs results in an increase in conjugated histamine, the increase being about 3 to 5 per cent of the free histamine fed. From the dog, they have obtained, in 24 hours, over 30 mg. of histamine (calculated as acid phosphate), and sometimes the concentration is as high as 100 γ/cc. of urine. The conjugated histamine has similarly been isolated from human urine. Subcutaneously injected conjugated histamine is almost quantitatively excreted. The oral administration of conjugated histamine leads to a rapid and much greater excretion of it in the urine than when histamine acid phosphate is fed. Between 50 and 60 per cent of the amount fed is excreted in the unchanged form.

This conjugated or combined histamine is freely dialysable and should be distinguished from the combined form of histamine, presumably with protein, which is nondialysable. Very little is known of the chemical nature of this conjugated form, nor is the site of its formation known, whether kidney, liver, or intestine. From what has been said, it will be appreciated that the elimination of histamine, in either the free or the combined form, into the urine plays only a small part in the removal of histamine from the body.

In 1929, Best[8] reported that naturally occurring and added histamine disappears during the autolysis of minced lung of the horse and cow. This work was extended and, in the following year, Best and McHenry[1] suggested that the substance or system which produces a change in structure responsible for the loss of biological activity of histamine be designated "histaminase" and the reaction be referred to as the "histamine-histaminase" reaction. Later, in 1938, Zeller[9] showed that a hog-kidney preparation not only oxidized histamine, but oxidatively deaminated a variety of other diamines, such as ethylene diamine, trimethyl diamine, putrescine, cadaverine, and agnatine, and also[10] a variety of substituted diamines, *e.g.*, spermine and spermidine. Zeller therefore recommended that the enzyme be called "diamine oxidase" rather than histaminase.

Diamine oxidase, or histaminase, is widely distributed in the animal kingdom. It has been found in cold-blooded animals, in birds, and in mammals. Recently, Roulet and Zeller[11] have reported a diamine oxidase from an acid-fast bacillus, with a very low activity towards histamine. It has been shown[12–14] that most mammalian kidneys, with the exception of those of the rodents, are rich sources of the enzyme. The cortex usually contains more activity than the medulla, except in man, where the enzyme content is approximately the same in cortex as in medulla. In the new-born child, the medulla has a much higher content of diamine oxidase than has the cortex. In most mammals, with the exception of the rodents, the kidney contains more histaminase than the liver, whereas, in birds, this relationship is reversed. The intestinal mucosa of practically all warm-blooded animals contains histaminase.

It was shown by Best and McHenry[1] that oxygen is necessary for the inactivation of histamine by histaminase. Several groups of workers since have attempted to correlate the oxygen consumption with the amount of histamine inactivated. Laskowski[15] clearly demonstrated that the rate of oxygen uptake varied with the purity of his preparation of histaminase. His purest preparation catalyzed the consumption of one atom of oxygen per molecule of histamine inactivated. It would appear that, with purer preparations of histaminase for each molecule of histamine destroyed, there is a usage of one atom of oxygen and the formation of one molecule of ammonia and one molecule of aldehyde. After prolonged incubation with cruder kidney extracts, the imidazole ring is ruptured, and all 3 nitrogen atoms of the histamine molecule can be set free as ammonia. On present evidence, it seems that the free amino group of the side chain is first removed, although Swedin,[16] working with a highly purified preparation (on present standards), considers the imidazole ring to be broken as a first step in the activation of histamine.

A variety of units of histaminase activity have been proposed. The original, proposed by Best and McHenry, was "that amount of activity which would destroy 1 mg. of histamine in 24 hours at 37°C. in a phosphate buffer at pH 7." A recent new unit is that proposed by Laskowski and his associates,[17] who claim it to be an advance over the older units because of the ease with which the unitage can be calculated from either the oxygen consumption or the liberation of ammonia. By definition, this new (Laskowski) unit is about 5.4 times larger than that of Zeller; 10 times larger than that of Stephenson;[18] 14 times larger than that of Best and McHenry; and 24 times larger than the "Torantil" unit.

Zeller considers histaminase to be a flavoprotein. He identified flavin after splitting the enzyme with acetone. Laskowski *et al.* were unable to confirm these findings. However, Kapeller-Adler[19] has prepared, from pig-kidney cortex, a powder possessing 1.5 to 2 units per mg. protein (Laskowski units). After dialysis during 1 to 3 days against tap water or phosphate buffer at pH 7.2, the activity of the preparation decreased towards histamine but increased towards cadaverine, putrescine, and agnatine as substrates. There was a loss of color of the enzyme preparation during dialysis, as in Zeller's experiments. When an acetone extract, yellow in color, of the original enzyme preparation was added to the dialyzed enzyme, the effects of dialysis were completely reversed. Kapeller-Adler has also been able to show that the prosthetic group of the flavoprotein, d-amino acid oxidase, namely, flavin adenine dinucleotide (F.A.D.), when added to dialyzed histaminase, was able to replace the yellow extract prepared from histaminase and equally well reversed the effects of dialysis. Further, the yellow extract from histaminase, added to the protein moiety of d-amino acid oxidase, was able to restore its activity. It appears, then, that the prosthetic group of histaminase is F.A.D. Kapeller-Adler suggests that F.A.D. may act as a coenzyme for the histamine-histaminase reaction but as an inactivator in the case of substrates such as cadaverine, putrescine, and agnatine, for F.A.D. (and the yellow extract) acts on undialyzed enzyme preparations, increasing the effect on histamine and decreasing the effect towards the other diamines used as substrates.

These experiments, quite apart from their innate interest, bring additional information with which to debate the nomenclature of this enzyme system. This work would seem to lend support to the retention of the term "histaminase" for these mammalian preparations rather than the more general term "diamine oxidase."

Marcou *et al.*[20] noted a considerable increase in the histamine-destroying power of the blood of pregnant women. This observation has been considerably extended, especially by Kapeller-Adler[21] and by Ahlmark,[22] who has also investigated similar changes in pregnant animals.

Ahlmark carried out his tests by a method in which the inactivation of histamine incubated with the plasma is measured biologically. Kapeller-Adler has used a biological procedure, but, in much of her work, she has used a modification of the test of Zeller *et al.*,[23] in which the extent of the decolorization of indigo disulphonate, presumably due to the intermediary formation of hydrogen peroxide, is used as the measure of enzyme activity, while employing histamine as substrate. It would appear that the accuracy and general reliability of this chemical method is inferior to that of a carefully controlled biological assay.

According to Ahlmark, the histaminolytic power (which he considers to be due to histaminase) begins to increase in the serum of the pregnant woman about the 7th week after the last menstruation and reaches a peak at the 7th month. It falls rapidly after parturition. It is suggested that the increased histaminolytic power of the plasma in pregnancy is due to the passage of the enzyme from the placenta.

Danforth[24] reported the presence of histaminase in the human placenta and later put forward the tentative suggestion that the amount of histaminase in the placenta showed some correlation with the efficiency of uterine contractions. Kapeller-Adler,[21] in her investigations of 45 placentae, again pointed to a possible correlation between the amount of the histaminase in the placenta having been found to be inversely proportional to the uterine efficiency.

Ahlmark found values of the histaminolytic power of sera deviating from normal in some cases of pregnancy toxemia. There appeared to be no correlation between the degree of symptoms and the histaminolytic power. Kapeller-Adler's results are not in agreement with those of Ahlmark. She favors the assumption that histamine might be formed, in the metabolism of the pregnant woman, from the histidine, present in large amounts throughout gestation, by the enzyme histidine decarboxylase. In normal pregnancy, most of this histamine is presumably destroyed by the histaminase. In mild cases of pre-eclamptic toxemia, the activity of the histaminase may be impaired and the histamine may cause damage. In severe cases of pre-eclamptic toxemia and in eclampsia, the histaminase activity is very low, and the histamine formed in considerable quantities will then exert its toxic effect, more particularly on the kidneys. The role of diamine oxidase, or histaminase, in the tissues is still uncertain; this present evidence is very suggestive.

The report of Karady and Browne,[25] that the intravenous injection of histaminase (Torantil) could inhibit histamine shock and anaphylactic shock in guinea pigs, has not been substantiated by later workers using larger doses of the same or of more potent enzyme preparations.[26–32] It should be added, however, that Lemley and Laskowski[33] were able to protect one-third of their guinea pigs, which they had injected simultaneously with the enzyme and with one and one-half times the lethal dose of histamine. They were unable to use more enzyme preparation because of its toxicity, though, by present standards, its activity per mg. of protein was high.

It would be out of place in this paper to discuss even briefly the clinical results of the use of histaminase preparations. One significant observation of Rose[34] may be mentioned: there was no effect on the histamine content of the blood of patients, following or during the administration of histaminase.

Stephenson,[35] in some preliminary studies, found that, when large doses of purified histaminase were injected intravenously into mice, it was possible to recover some of the enzyme in their tissues. A mouse receiving 65 units intravenously was killed 5 minutes later, and 90 per cent of the activity was recovered. When 130 units were given and the mouse killed one hour later, 50 per cent of the activity was recovered. The tissues of the mouse examined were blood, liver, and kidney. The enzyme was found in approximately equal amounts in liver and blood. None was recovered from the kidney. Stephenson presumed that the liver destroyed the injected enzyme.

Dale[36] first showed that adrenalectomy greatly enhanced the sensitivity of animals to injections of histamine. Many later workers have focused their attention on the part played by the adrenal cortex in controlling the effects of histamine, though it has been shown that the medulla, with its secretion of adrenaline, is also involved. The hypophysectomized animal is also more sensitive to histamine than is the normal animal, and, presumably, this difference may be ascribed to the accompanying atrophy of the adrenals. MacKay and Clark[37] found that the adrenal glands of rats given daily subcutaneous doses of histamine weighed 30 per cent more than those of controls and that the hypertrophy was confined to the cortex. A number of other experimenters, while producing a tolerance to histamine by repeated daily injections, have not been able to observe any increase in weight of the adrenal glands. It might be interjected here that the adrenals play only a minor role in the development of both tachyphylaxis and a tolerance towards histamine. A change in surface membrane conditions, as suggested by the experiments of Schild,[38] may well provide an explanation for these states (see also Dale[39]).

It has been shown by a number of independent workers that the resistance to histamine of the adrenalectomized animal may be restored to normal by the administration of cortical extract, though, as Rose[40] showed, considerably more than the maintenance dose of cortin for the adrenalectomized rat is required to restore the full ability of the animal to inactivate histamine. Karady *et al.*[41] observed a decrease in the histaminase content of the lung of adrenalectomized rats and that the enzyme could be restored to normal with adequate amounts of cortin. The histaminase content is consideraby decreased if normal saline is not given in the diet of the adrenalectomized rats. When they are given normal saline, however, the enzyme content is only slightly decreased, and yet the rats are markedly sensitive to histamine when compared with the normal. It does not seem, as these workers pointed out, that the decreased resistance is due to the decrease in the histaminase, nor is it linked to a specific function of the adrenal glands. It is only one example of the generally decreased resistance of the organism under these conditions (see also Swingle and Remington[42]).

When anaphylactic shock was superimposed on histamine shock (in the rabbit[43]), the removal of histamine was faster than when histamine alone was given, suggesting that some mechanism for the rapid destruction of histamine must be activated by the antigen-antibody reaction.

Rose and Browne[44] carried out an investigation on the rate of disappearance of intravenously injected histamine in the rat. In some experiments, 24 γ/g. body weight were given and a succession of animals was killed at varying intervals of time after the injection. Within 15 minutes, relatively little histamine remained in the blood, the concentration of histamine being far higher in other tissues examined, namely, kidney, liver, lung, and lymph glands. The histamine content of the kidneys was particularly high, being 1600 times its normal value. In the interval between 15 and 30 minutes after injection, there was a sharp decrease in the histamine content of the liver. In the kidney, the decrease was only slight. Within 3 hours, all values approached the normal except those of blood and kidney. These authors suggested two possible explanations for the difference in the rate of disappearance of histamine from the various tissues studied. Assuming the presence of some mechanism in the kidney, other than histaminase, which is not present in the kidney of the rat, the fall of the concentration in the lung, liver, *etc.*, during the 15 to 30 minute period, and the absence of any decrease in the kidney are due to the continued transfer of the histamine from the other tissues to the kidney at such a rate that the destruction of histamine by the kidney is not apparent. If, however, one assumes that the main mechanism of histamine destruction is the histaminase action of those tissues which contain it, namely, lung and small intestine, the kidney in this case could be regarded as an organ which takes up a large amount of histamine, thus temporarily removing it from the circulation and slowly liberating it to be transferred to the histaminase-containing organs for destruction.

In summary, at present it would appear that the chief mechanism brought into play, as soon as there is an increase in the concentration of free histamine in the extracellular fluids of the body, is the direct attachment of histamine to cells, presumably to protein molecules of the cells which thereby anchor the histamine and temporarily render it inactive. Later, one presumes that the histamine is either inactivated, in the tissue to which it has become attached, or it is reversibly attached and, as the concentration of free histamine decreases in the surrounding fluid, it is carried to other inactivating tissues, which may derive at least some of their power of inactivation from their content of histaminase. It may be that histamine is also inactivated by mechanisms as yet unidentified.

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Discussion

C. A. Dragstedt (*Northwestern University Medical School, Chicago, Illinois*): There is a point in connection with the inactivation of histamine, which Dr. Waters has presented so ably, that merits mention because of its historical interest. A number of investigators, including Richard Weil in particular, made serious efforts to detect an anaphylatoxin (such as histamine) in canine anaphylactic shock by transfusing the blood of anaphylactic animals into normal animals. In view of the fact that anaphylactic reactions in the dog do not occur with an assured and predictable severity, they felt it necessary to wait about thirty minutes after the assaulting injection of antigen before withdrawing blood, in order that the effects of the blood-letting alone would not becloud or confuse an estimation of the severity of the anaphylactic reaction.

As we have since learned, this lapse of time was adequate for the complete disappearance of free histamine from the circulating plasma. As it is only the free histamine in the plasma which exercises physiologic effects, the negative results of such experiments are readily understood. In contrast, the relative ease with which we were able to demonstrate the liberation of histamine during anaphylaxis in the dog was due to the method which we first employed. Lymph was collected continuously from the thoracic duct, and, as histamine is inactivated at a negligible rate by lymph *in vitro* (or by dog's blood plasma *in vitro*, for that matter), our very first experiments were crowned with success. A similar explanation obtains for the positive results which Bartosch, Feldberg, and Nagel achieved when they perfused the lungs of anaphylactic guinea pigs and detected a histamine-like substance in the perfusate. It was only after there was due appreciation of the astonishing speed with which free histamine disappears from the circulating blood that it was possible to demonstrate substantial amounts of histamine in the blood of these animals during anaphylaxis.

HISTAMINE-PROTEIN COMPLEXES IN ANAPHYLAXIS AND ALLERGY. A REVIEW

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The synthesis of histamine-azoprotein was attempted several years ago in an effort to discover if a histamine-protein complex could be used to create an artificial immunity to histamine in animals and man. If histamine-specific antibodies could be developed, it was postulated that they might bind any histamine released during an antigen-antibody reaction *in vivo*. The bound histamine might not be able to reach or affect the shock organs, and thus some of the typical symptoms of anaphylaxis and allergy might be prevented. This hypothesis was based, of course, on the original important observations of Dale and Laidlaw,[1] as well as those of Lewis,[2] and the fundamental work of Landsteiner in the field of immunologic specificity.

The method of synthesis and the immunologic properties of several histamine-azoproteins[3, 4] (differing only in the protein components) and of a histamine-isocyano protein[5] have been published. This work was interrupted by the war and has been resumed only in a limited form. Some of the published material and certain earlier work which did not reach a stage justifying publication will be described in this review.

The synthesis of the first histamine-azoprotein presented some difficulties, but these were finally overcome and the compound was obtained in the desired form. The details of the synthesis have been described previously.[4] The protein used most frequently was despeciated normal horse-serum globulin prepared by the Taka-diastase antitoxin concentration process of Coghill and Fell.[6] This protein was employed so as to eliminate, as far as possible in clinical studies, any risk of sensitization to intact horse-serum protein, in case the whole theory of effective immunization by histamine-azoprotein proved to be unsound. Despeciated normal horse-serum globulin has had most of its horse-serum specificity removed by the treatment with Taka-diastase.

In laboratory experiments, other proteins frequently were used for coupling. These included casein, normal horse-serum globulin, cow-serum globulin, and dog serum. The histamine-azoprotein solution was diluted to contain about 2 per cent of the complex, the pH adjusted to 7.6, phenol added to a concentration of 0.5 per cent, and the solution sterilized by Mandler filtration. The amount of bound histamine in such a solution was usually about 1 mg. per ml. The histamine in the final product was firmly bound and no tests for free histamine were ever positive. The bound histamine could be liberated, however, by hydrolysis of the product with concentrated hydrochloric acid. After such hydrolysis the liberated histamine could be assayed by a method such as that of Code.[7]

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* The investigations described in this paper were carried out in the Research and Biological Laboratories of Parke, Davis & Company, Detroit, Michigan.

The immunologic specificity of histamine-azoprotein was tested mainly with antisera from rabbits hyperimmunized with histamine-azo-despeciated-horse-serum globulin. Antisera with fairly high precipitin titers could be obtained only by immunizing rabbits intensively by intravenous injection for a period of 6 to 8 weeks. The observed weak antigenicity of the complex is in agreement with observations by Landsteiner and VanderScheer,[8] Hooker and Boyd,[9] and others that basic haptens such as histamine are inferior to acidic in the production of specific antisera. Results of a typical precipitative test with rabbit antiserum are shown in TABLE 1 and, as can be seen, definitely positive tests usually were obtained only at antigen dilutions not higher than 1:800. However, most of these rabbit antisera produced cross reactions with histamine-azoproteins made with heterologous protein components. Results of a typical test of this type are given in TABLE 2. Precipitation with the homologous protein was also obtained, indicating that the specificity of this protein had not been completely altered

TABLE 1

PRECIPITATIVE TESTS WITH RABBIT ANTISERUM TO HISTAMINE-AZO-DESPECIATED-HORSE-SERUM GLOBULIN

Rabbit no.	*Antigen dilutions*							*Normal rabbit serum 1–50*
	1–10	*1–50*	*1–100*	*1–200*	*1–400*	*1–800*	*1–1600*	
1	++++	+++	+++	++	+	+	±	–
2	++++	+++	++	+	+	+	±	–
3	+++	++	++	++	+	+	±	–
7	++++	+++	++	++	+	+	–	–
8	++++	+++	++	++	++	+	+	–
10	+++	+++	++	++	+	+	±	–

0.2 ml. of the antigen dilution and 0.2 ml. of the antiserum mixed, left for 1 hour at 37C., 18 hours in the refrigerator, and read.

TABLE 2

CROSS PRECIPITATIVE REACTIONS OF ANTIHISTAMINE-AZO-DESPECIATED-HORSE-SERUM-GLOBULIN-RABBIT-SERUM WITH VARIOUS HISTAMINE ANTIGENS

Dilution of Antigen	*Antigens Tested*			
	Histamine-azo-despeciated-horse-serum globulin	*Histamine-azo-rabbit serum*	*Histamine-azo-casein*	*Despeciated horse-serum globulin*
1–80	+++	++	+±	++
1–160	++	+±	+	+
1–320	+±	+	–	+
1–640	+	–	–	–
1–1280	–	–	–	–

Controls = antigen + saline
antigen + normal rabbit serum
antigen + normal horse serum
immune serum + saline
} negative
All antigens prior to dilution contained 5–6 mg. N per ml.

by combining it with para-aminobenzoyl histamine. The results of the inhibitive test shown in TABLE 3 give another indication of the partial specificity of antibodies produced by histamine-azoproteins. These results show inhibition of precipitin reaction by histamine and some inhibition by para-aminobenzoyl histamine and histamine azocasein. Compounds closely related chemically, such as tyramine and histidine, however, did not have any inhibitive capacity.

A study of protection against anaphylaxis by prior immunization with the histamine-protein complex was carried out in guinea pigs and rabbits. The procedure consisted of immunization of a series of animals with the complex, followed by sensitization to crystalline ovalbumin. A series of controls was also sensitized, following which all the animals were injected with shocking doses of ovalbumin.

Some results of the direct anaphylaxis experiment with guinea pigs are shown in TABLE 4. Immunization consisted of three subcutaneous injections weekly of histamine-azoprotein: 0.5 ml. the first week, 1.0 ml. the second, and 1.5 ml. the third week. The control animals were treated similarly with equivalent amounts of despeciated horse-serum globulin.

TABLE 3

INHIBITIVE TESTS WITH RABBIT ANTIHISTAMINE-ANTIGEN SERUM

Time	*Precipitative reaction after incubation with inhibitors*											
	Saline		*Histamine HCl*		*P-NH$_2$ benzoyl histamine*		*Tyramine HCl*		*Histidine HCl*		*Histamine-azo-casein*	
			20 mg.	*10 mg.*	*20 mg.*	*10 mg.*	*20 mg.*	*10 mg.*	*20 mg.*	*10 mg.*	*10 mg.*	*5 mg.*
minutes												
30	3+	3+	–	2+	–	3+	3+	3+	+	+	–	+
50	4+	4+	–	3+	+	4+	3+	4+	2+	3+		
70	4+	4+	–	4+	3+	4+	4+	4+	3+	4+		
120	4+	4+	–	4+	4+	4+	4+	4+	4+	4+		

0.2 ml. antiserum 1:28 incubated with 0.2 ml. inhibitors at 37 C. for 1 hour and 0.4 ml. of histamine-azo-horse-serum-globulin then added.

Both series were then sensitized with three doses of 5 mg. of crystalline ovalbumin given subcutaneously at two-day intervals. One day after the last albumin injection, the treated series received an injection of 1 ml. of histamine azoprotein and the controls were given an equivalent amount of despeciated, normal horse-serum. One week later, this immunizing dose was repeated. After four weeks, all the animals were given shocking doses of albumin. It is evident that some definite protection against anaphylactic shock is produced by this treatment. Statistical analysis showed that the differences in anaphylactic reaction between the two groups were significant.

Anaphylaxis in rabbits immunized with histamine-azoprotein and sensitized to ovalbumin was investigated by checking blood pressure before and during the period of shock produced by intravenous injection of ovalbumin.

The blood pressure effects were not particularly significant, although, in the control group, 5 of 8 rabbits had a pronounced drop below normal and there were 3 deaths. Four of 6 immunized animals showed only a slight rise in pressure with slow return to normal; the other two exhibited little or no change in blood pressure.

Table 4

Anaphylaxis in Guinea Pigs Immunized With Histamine-Azoprotein and Subsequently Sensitized to Ovalbumin

Control animals		*Histamine-azoprotein-treated animals*	
Crystalline ovalbumin, 10%	*Anaphylaxis*	*Crystalline ovalbumin, 10%*	*Anaphylaxis*
ml.		*ml.*	
A 1.0 I.A.	++	2.0 I.A.	±
1.5 "	+++	2.0 "	±
2.0 "	++++	3.0 "	±
2.0 "	+++	3.0 "	±
3.0 "	++++	3.0 "	++
3.0 "	++++	3.0 "	+
3.0 "	++++	3.0 "	+
3.0 "	++++	3.0 "	+
B 5.0 "	++	5.0 "	++
2.0 "	++	0.5 I.V.	+
2.0 I.V.	++++	1.0 "	++
1.0 "	++++	3.0 "	++
C 1.0 I.A.	++	3.0 I.A.	±
2.0 "	++++	3.0 "	±
2.0 "	++	3.0 "	±
3.0 "	++++		
3.0 "	++++		
3.0 "	++++		
2.0 "	++		
50% fresh albumin		*50% fresh albumin*	
D 5.0 I.A.	++++	4.0 I.A.	+++
3.0 "	++	4.0 "	++++
4.0 "	++++	4.0 "	++++
4.0 "	++++	4.0 "	+++
4.0 "	++	4.0 "	+++
4.0 "	++	4.0 "	+++
5.0 "	±	4.0 "	+
4.0 "	+++	4.0 "	+
		4.0 "	+

++++ = fatal anaphylaxis with typical symptoms and pathological findings on autopsy.
I.A. = intra-abdominally; I.V. = intravenously.

Blood was taken from the hearts of immunized and sensitized rabbits prior to the administration of the shocking dose of ovalbumin, and this blood was used for experiments on *in vitro* liberation of histamine according to the method of Katz.[10] A summary of all experiments of this type showed that the plasma of 13 of 17 control animals yielded one microgram per ml. or more of released histamine, while in the plasma of only 3 of 17 immunized animals was there a similar increase in histamine. These differences were also shown to be significant by statistical analysis. The trypan-blue test that has been employed by Ramsdell[11] and Rocha e Silva and Dragstedt[12] for the identification of histamine liberation in tissues was also employed with rabbits immunized to histamine-azoprotein and subsequently sensitized to ovalbumin. In a small series of animals, normal controls sensitized to ovalbumin gave good positive trypan-blue results when tested with ovalbumin and also with histamine, whereas the immunized rabbits showed negligible histamine release on injection of ovalbumin and a considerably smaller response to histamine than was the case with the controls.

The partial specificity for histamine of antibodies to histamine-azo-despeciated-horse-serum globulin has been confirmed by Rosenman and Starin,[13] using laboratory animals, and also to some extent by Cohen,[14–17] with human sera from patients treated with the histamine-protein complex. Using the iontophoresis technique with mixtures of histamine and serum from treated patients, he frequently could demonstrate that considerably lower dilutions of histamine were required to produce whealing than was the case with histamine in saline or normal serum. Where the threshold to histamine was normally 1:640,000, dilutions of the compound in treated serum necessary to produce wheals varied from 1:64,000 to the normal dilution. Two of these antisera were sent to Katz,[18] who reported that one of them neutralized 25 per cent of added histamine base when tested by his method on the guinea-pig ileum. Cohen, using 7 human sera and testing for precipitins by the Goodner method, found that all but one were 2 to 4 plus at 1 to 160, and 4 of the 7 were 3 or 4 plus at 1 to 640. One of these sera also gave 3 plus precipitation at a dilution of 1 to 128 with histamine-azocasein. With some of these human sera the inhibition reaction could be demonstrated.

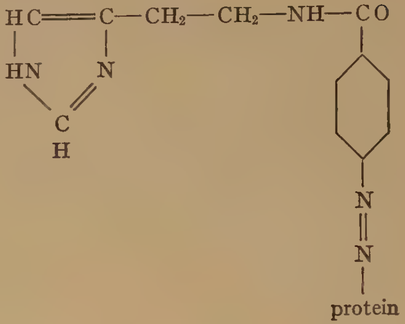
However, in all the clinical and laboratory reports that have accumulated in the literature, the relatively poor antigenicity of histamine-azoprotein has been evident. Large dosages at frequent intervals for a comparatively long period of time have been necessary in order to produce demonstrable antibodies in animals and man.

One of the interesting aspects of immunization with histamine-azoprotein is that the antibodies produced do not seem to interfere in any way with the normal functions of histamine in animals or man. Laboratory and clinical studies have indicated that immunization with this compound has no effect on gastric secretion, capillary dilatation, or blood pressure. Similar results were obtained by Clutton[19] with thyroxin globulins and by Went[20] with adrenalin azoprotein. None of the results reported by these workers indicated any effect on the normal function in the animal body of the chemical agent that was acting as a hapten in the complex antigen.

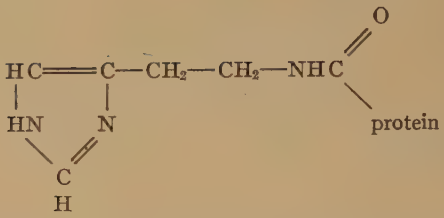
Other types of histamine-protein complexes have been prepared, and details of the synthetic methods described.[21] None of these complexes other than H-isocyanoprotein was tested immunologically, but it might be interesting for someone to investigate them, particularly those where protein was coupled with histamine at some point other than through the free amino group. A list of these complexes, including histamine-azoprotein, is given in TABLE 5.

TABLE 5
HISTAMINE-PROTEIN COMPLEXES

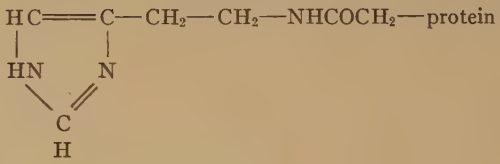
Histamine-azo-despeciated-horse-serum-globulin



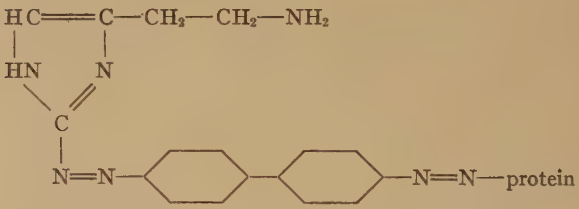
Histamine-isocyano-protein



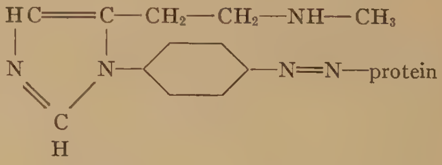
Complex from chloracetyl histamine



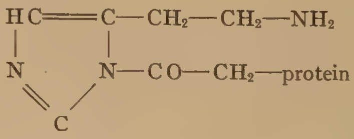
Complex from 2-(azobenzidine azo proteo-) histamine



Complex from 5-(β-methylaminoethyl-)-1-imidazyl-p-benzene diazonium chloride



Complex from 5-(β-carbobenzoxy-amidoethyl)-1-chloracetyl imidazole



The clinical reports[3, 14, 16, 17, 22–32] on histamine-azoprotein indicate a marked discrepancy in the results obtained. A number of physicians have reported using the compound with excellent results in cases of atopic eczema, contact dermatitis, urticaria, physical allergy, allergic rhinitis, histamine headache, allergy to flea bites, and sensitivity to liver extract. On the other hand, some clinicians have reported little or no benefit from the use of the compound. Furthermore, some fairly severe local and a few systemic reactions have been reported during recent years following the injection of histamine-azoprotein. This is somewhat surprising, because, during all the early clinical work with the compound, practically no reactions were seen other than mild local reactions and an intense area of local redness when the complex was injected intracutaneously. In no case was there a reaction which might be ascribed to sensitization to histamine-azoprotein itself. There were 2 or 3 instances where patients experienced some degree of what appeared to be histamine shock almost immediately after injection of the complex. It was felt that certain individuals might have a peculiar enzyme system that could liberate histamine from the complex. It seems possible that some of the later reactions reported may have been of this nature.

During recent years, attempts have been made to increase the antigenicity of histamine-azoprotein. These involved combinations of the complex with adjuvants and also adsorption of histamine-azoprotein on alum, calcium phosphate, or aluminum phosphate. Immunization with these materials usually shortened the time required for precipitins to appear in rabbit serum, but in no case was the precipitin titer raised appreciably over that which could be obtained with histamine-azoprotein itself. Another method tested involved the addition of more tyrosine by coupling on the acid azide to despeciated normal horse-serum globulin. This compound did not give evidence of any increase in antigenicity.

It was found recently that despeciated normal horse-serum globulin had considerably more tyrosine than did the untreated protein. The comparative amounts were 21 and 6.7 per cent. On this basis, therefore, it theoretically should be possible to attach more histamine to the protein molecule than had been assumed initially, and this proved to be true. However, the histamine complex, with considerably greater histamine content than the usual compound, was not improved in antigenicity.

Coffin and Kabat[33] have conducted an interesting investigation on histamine-azoprotein, using passively sensitized guinea pigs in protection experiments. They found that guinea pigs immunized with the complex showed little or no protection against 1 MLD. of histamine injected intravenously. We had made a similar observation, but we did notice some slight protection in immunized rabbits, as judged by the decreased fall in blood pressure, compared with that of control animals following the intravenous injection of histamine. We assumed that the lack of protection against histamine could be explained, as Coffin and Kabat have suggested, on the basis that histamine released during anaphylaxis is developed more slowly, at different sites, and in different quantities from that injected intravenously.

It seems probable that intravenous injection permits histamine to reach the organs of shock before there is time for antibodies to neutralize it.

Coffin and Kabat also found that guinea pigs immunized with histamine-azoprotein were protected against subsequent anaphylaxis, but that control animals immunized with human serum were similarly protected. This is an interesting finding in view of the fact that our control animals in anaphylaxis experiments with guinea pigs were always immunized with despeciated normal horse-serum globulin equivalent to that contained in the complex given the treated guinea pigs. Our control group received practically no protection from the protein itself. Of course, we were dealing with active sensitization, whereas the experiments of Coffin and Kabat involved the passive method. It would be interesting if their experiments could be repeated using actively sensitized guinea pigs.

Coffin and Kabat have also reported, on the basis of 4 guinea pigs, that active sensitization to histamine-azoprotein was possible. No control animals were mentioned. In our experience, we never could produce anaphylaxis in guinea pigs actively sensitized to the complex until we reached a dosage that intravenously would kill normal guinea pigs. We assumed, therefore, that deaths occurring in both sensitized and normal animals were really anaphylactoid in nature.

A survey of all the literature that has accumulated about histamine-azoprotein leads to the conclusion that the compound has helped support the theory that histamine plays some role in anaphylaxis and allergy. The results obtained with the antihistamine drugs, such as Benadryl, Antergan, and Pyribenzamine, have almost forced acceptance of the histamine theory, at least to the extent that histamine almost certainly must be considered one of the factors involved in anaphylactic and allergic reactions. A summary of all the clinical reports that are available about the antihistamine drugs and histamine-azoprotein indicates that the percentage of beneficial clinical results is approximately the same for all these compounds. Moreover, all of these antihistamine agents seem to be most effective clinically in the same types of allergies, namely, urticaria, atopic dermatitis and eczema, vasomotor rhinitis, and physical allergies, while none seems particularly beneficial in bronchial asthma. It seems possible that, in certain types of allergy, histamine may play a more dominant role than in others, but it is also likely that, in practically all forms of allergic reactions, there are other factors involved in addition to histamine.

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Discussion of the Paper

R. A. Cooke (*Roosevelt Hospital, New York, N.Y.*): My remarks will be directed primarily toward the use of histamine azoprotein by physicians in clinical allergy rather than to immunochemical aspects.

The theory on which Dr. Fell[1, 2] and his associates proceeded in the preparation of histamine azoprotein was an interesting one, for, while there is still no absolute proof, evidence is accumulating that histamine probably is liberated in anaphylactic reactions as one of the active substances responsible for the symptoms. Purely by analogy, therefore, it may be important also in certain of the clinical allergies. To this point I shall return later, for all allergies are not identical.

Dr. Fell states that the clinical reports indicate a marked discrepancy in results obtained. Actually, the number of authoritative clinical reports are few, although it is known that the commercial preparation, Hapamine, was extensively used by physicians generally. After several years of study, Sheldon *et al.*,[3] reporting on thirty-nine cases, were able to say no more than that the results were "sufficiently encouraging to justify a continuation of the study." In 1943, Cohen and Friedman[4] presented serologic studies of a few patients treated with histamine azoprotein, but did not pretend to furnish evidence of any therapeutic effect. Their last contribution[5] on histamine azoprotein therapy, however, was limited to fifty-five cases, with 50 per cent negative results. Dundy, Zohler, and Chobot[6] concluded that "treatment with histamine azoprotein was generally ineffective in a series of forty allergic individuals." A small therapeutic trial with serologic studies, conducted at my clinic at Roosevelt Hospital with Hebald and Downing,[7] led us to conclude that histamine azoprotein did not protect patients with pollen hay fever, did not prevent the induced constitutional reactions (analogue of anaphylactic shock) in ragweed-sensitive patients, and did not alter the level of the cutaneous reaction on intradermal test to the histamine base. Although precipitins to the histamine azoprotein were found in the blood of five of eight patients, in no case could we demonstrate a precipitate to casein-azoprotein. This I believe to be the extent of published clinical studies. They are very meager and, as far as they go, do not support a claim for very beneficial therapeutic effects. Further, the rapidity with which this compound has disappeared from the therapeutic arena would indicate that it proved useless for treatment of allergies in the hands of the medical profession generally.

There is another aspect, however, which I feel warrants mention. Granted that histamine may be a factor in allergies of the immediate wheal type, the one most likely based upon a mechanism similar to that of anaphylaxis (*e.g.*, hay fever, urticaria, and certain of the asthma group), one must, I believe, differentiate sharply between these and those allergies of the so-called "delayed group," in which belong dermatitis of external or internal origin, certain vascular reactions, and the tuberculin type of bacterial allergy. The reasons are: (1) the histologic response in these latter reactions bears no relation to the pharmacologic effect which histamine is known to produce in tissues; and (2) the antibody mechanism is different.

Bearing this point in mind, if we again review the recorded studies, it appears that a large number of the treated patients exhibited allergies of the delayed type. It was in them that most of the best results were obtained by Sheldon *et al.* and by Cohen and Friedman. As a matter of fact, the latter authors state "it (histamine azoprotein) is of no value when used alone in the prevention of hay fever" (which is typical of the immediate allergies that might be produced by liberation of histamine). The interpretation of therapeutic results is hazardous business, and the results just referred to and attributed to the drug used (histamine azoprotein) merely indicate to my mind the difficulties and fallacies inherent in clinical studies generally.

It needs only to be mentioned that histamine azoprotein is antigenically active, as proven by Coffin and Kabat,[8] and not devoid of danger in the treatment of patients.

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S. B. Hooker (*Boston, Massachusetts*): One aspect of this topic that has not been given adequate consideration is the neutralizing strength of antihaptenic sera which can be produced by immunization with proteins coupled with pharmacologically active substances of low molecular weight. About fifteen years ago, Dr. Boyd and I attempted unsuccessfully to produce antimorphine. Later, we did obtain several antisera from rabbits prolongedly immunized with a strychnine-hemocyanin-diazonium compound. These sera were weak but specifically precipitated with casein-azo-strychnine. This aggregative property was specifically inhibited by various salts of strychnine and by brucine, but the sera failed entirely to protect mice against a single lethal dose of strychnine.

During that period, we considered the possibility of producing a specific antihistaminic serum for the purpose of combating shock, but a simple calculation dissuaded us from making the attempt. These azoproteins are poor antigens. With us and with others, the amount of antihapten they had produced was measured in micrograms rather than milligrams. When we made the liberal assumption that sera ten times stronger than ours might be obtained and, further, over-liberally allowed *quadri*-valence to each molecule of antihapten, as well as irreversible combination, computation revealed that about three liters of immune serum would be required to neutralize a single average dose of morphine lethal to man. It is well known, of course, that a relatively enormous number of simple haptenic molecules is required to inhibit antihaptenic sera, and this indicates a high dissociation constant, probably increasing as the molecular size (and complexity) of the hapten diminishes. Histamine is about one third as large as morphine.

For these and other reasons, the production of an antihistaminic serum did not promise much in the way of practical usefulness, which, however, does not derogate from its theoretical interest.

INTRODUCTION TO ANTIHISTAMINE AGENTS AND ANTERGAN DERIVATIVES

By Daniel Bovet

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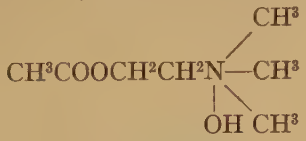
The scope of therapeutic chemistry can be characterized by the problem it intends to solve, which is essentially the study of the relationship between molecular structure and physiological activity. This field of chemical therapeusis may be represented by a large checkered table, with the pharmacodynamic properties of the agents under consideration being placed on the axis of ordinates and the long list of known chemical products on the axis of abscissas. It may be seen at once that, in either direction, developments are practically unlimited.

In the present state of our knowledge, it is possible, in certain fields of pharmacology, to evaluate the data so obtained and thereby establish certain methods of approach which may be applied in a general way. I intend to illustrate these various methods of approach, selecting as an example the history of the investigations on antihistamine substances, a subject which has demonstrated with particular sharpness how integrated have been the efforts of pharmacologists and chemists.

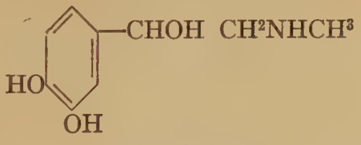
These are the points to be illustrated in this paper. A report will be made on the results of investigations carried out since 1937 by numerous research workers, in various countries, on antihistamine substances.

Antihistamine drugs[1] represent a group of synthetic amines displaying in most animals species, as well as in man, an antagonism specifically directed against the physiological and toxic effects of histamine. Perhaps no better definition could be given of an antihistamine substance than to state that it is a counterpoison having no specific activity of its own on the normal animal, its properties appearing only when it can manifest a detoxifying power against the action of histamine. The great activity of antihistamine agents is not due to any spasmolytic, sympathomimetic, or analeptic properties. In experimental pathology and human therapeutics, antihistamine substances are active against a large number of disturbing manifestations, particularly against anaphylactic reactions, which in many instances have been attributed to the release of histamine in the tissues.

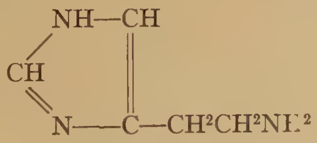
Three naturally occurring amines, acetylcholine, epinephrine, and histamine, may be grouped together—



acetylcholine



epinephrine



histamine

—because they have a similar chemical structure, are all present in the body fluids, and exert characteristically strong pharmacologic activities.

There are alkaloids which interfere with the effects of acetylcholine. Similarly, there are sympathicolytic poisons which neutralize or reverse the effects of epinephrine. It seemed possible to me, therefore, that some substance might exist which exerts a specific antagonism toward histamine.

With this hypothesis in mind, I began an investigation in 1937 to determine the effect upon the susceptibility toward histamine of various substances of known activity on the autonomic nervous system. The results obtained with a series of phenolic ethers, including substances which possessed either antiepinephrine or antihistaminic activity, led to the first confirmation of the validity of this assumption. Since then, compounds with similar or greater activities have been found in approximately ten different chemical series.

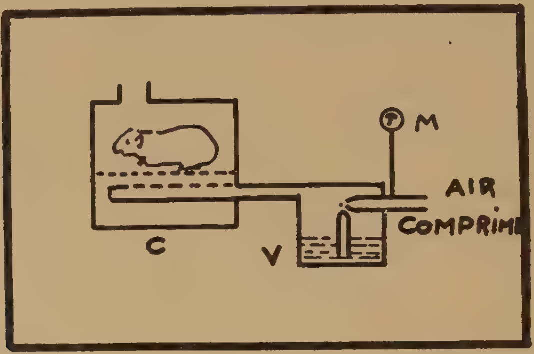


Figure 1. Diagram of an apparatus in which bronchospasm in guinea pigs is produced by inhalation of histamine aerosol. C—closed box in which the animal is observed; V—aerosolizer containing a solution of histamine hydrochloride; M—manometer (Bovet and Walthert[31]).

A certain number of laboratory methods have been proposed for the evaluation of the degree of activity of the various compounds. In the first test, we determined the action against the lethal effects of histamine in guinea pigs. This test is perfectly specific. In the second test, we determined the protection against histamine administered in the form of an aerosol. Here, symptoms similar to asthma are produced (FIGURE 1). A third method for determining antihistaminic activity, the least specific one, consists in ascertaining, *in vitro*, the effect on histamine-induced spasm of the isolated guinea-pig ileum.

First of all, it is important to note that many substances with a widely differing chemical constitution exhibit antihistamine properties, but that only few substances present this property to a high degree. Thymoxyethyldiethylamine, or 929 F,* is the first of the substances in which antihistamine properties were recognized.

From the chemical standpoint, Compound 929 F belongs to a series of amines with a phenolic ether function. It is generally known that it was

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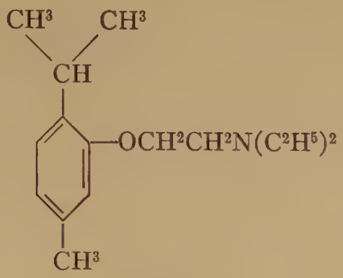
* F = Fourneau.

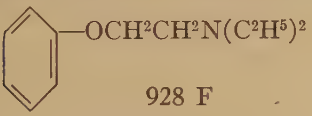
Fourneau (chief of the Department of Therapeutic Chemistry in the Pasteur Institute, and with whom I had the privilege of close association for twenty years), who, in 1910, was one of the first to synthesize the amines of this group.[2]



929 F

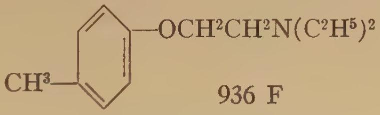
Eichholz,[3] in 1928, called attention to the properties of Gravitol, and Anan,[4] in 1929, described similar properties of Tastromine or thymoxyethyldimethylamine. Since 1933,[5] further derivatives were prepared in France, where particular attention was drawn to the sympatholytic activity of Prosympal (883 F[6]). Other derivatives of the same group are sympathomimetic or nicotinic or else they exert an activity which mainly influences the heart.[7, 8]

In 1937, a thorough investigation of 929 F, carried out with Staub, who was then preparing her doctorate thesis in my laboratory, made it possible to show: (1) the protection conferred to guinea pigs which had received several lethal doses of histamine;[9] (2) the antagonism directed against the histamine-induced spasms of smooth muscles of the intestine, stomach, uterus, and bronchi;[9, 10] and (3) the protection against experimental anaphylactic shock.[11] These first results have been confirmed and enlarged by several authors, more especially in the United States by Rosenthal and



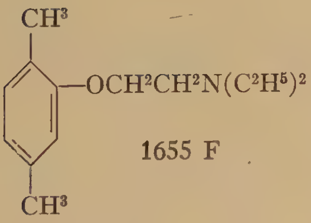
928 F

Protection: none



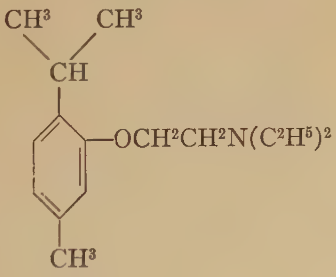
936 F

Protection: none



1655 F

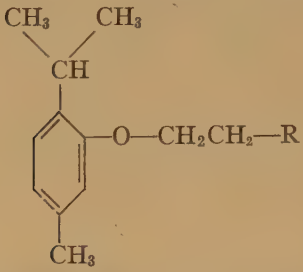
Protection: 2 doses



Protection: 3 doses

Minard,[12] Brown,[13] Lambert,[14] Climenko, Hamburger, and Messer,[15] Burchell and Varco,[16] Loew and Chickering,[17] Bourque,[18] and Hallenbeck.[19]

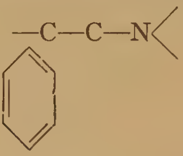
Thymoxyethyl-diethylamine (929 F) is the most interesting substance of antihistaminic activity within the phenolic ether series. Staub[10] has shown that antihistamine activity is strongly influenced by substitutions on the nucleus. Tastromine, a dimethylated homologue of 929 F., is probably somewhat more active. On the other hand, the corresponding derivative with a secondary amino group is totally ineffective.

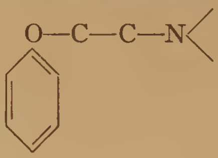


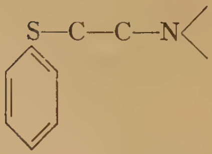
NHC_2H_5 R	Protection: none
$N(CH_3)_2$ Tastromine	4 doses
$N(C_2H_5)_2$ 929 F	3 doses

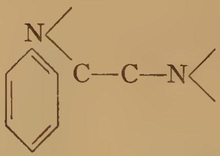
Through the experimental research on thymoxyethyl-diethylamine, it was possible to ascertain for the first time that there exist substances which exhibit antagonistic activity towards histamine.

Previous studies of sympathomimetic and sympatholytic poisons, carried out in the Pasteur Institute, had shown that a relationship exists in the pharmacodynamic responses between the different series of alkyl-aromatic amines: phenylethylamine, phenoxyethylamine, phenylthiothylamine, and phenylethylenediamine.[8] It seemed obvious, then, to apply our improved techniques of antihistamine assay to these series. Since neither phenylethylamines nor thioethylamines counteracted histamine, we turned to the study of aniline compounds. Derivatives of aminoethylaniline had been the object of limited study before we had found their antihistaminic activity.



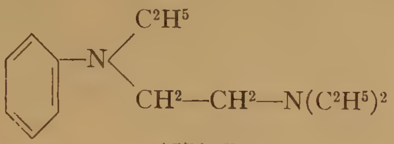




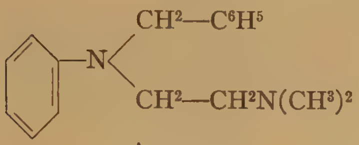


A compound of this type is described in a work by Weese[20] dealing with analeptic research on Icoral. Its cardiotonic action is mentioned in a paper by Pratesi.[21] Synthetic research undertaken in Fourneau's laboratory by de Lestrange and J. P. Fourneau[22] has disclosed that, in this series, sympathomimetic, sympatholytic, and antihistaminic amines are found side by side.

One derivative, diethylaminoethyl-N-ethyl-aniline, (designated 1571F), has proven to be more effective than 929F.[10] Our results published in 1939 have been confirmed in America by Burchell and Varco,[16] Loew,[17, 23, 24] Bourque,[18] Wilcox and Seegal,[25] *et al.* and in France by Halpern[26] in 1940, 1941, and 1942.



1571 F

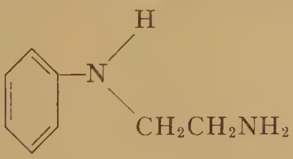


Antergan

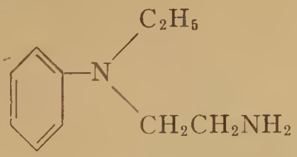
In 1942, a dimethylamine derivative corresponding to 1571F, N-diethylaminoethyl-N-benzyl-aniline (Antergan), was prepared by Mosnier and investigated by Halpern.[26] Utilizing the aforementioned toxic-dose test in guinea pigs, he showed that this derivative was approximately ten times more active than 1571F. Antergan, now replaced in France by Neoantergan, was the first antihistamine agent commonly employed in therapy.

Considerable data are now available on the relationship between pharmacodynamic activity and chemical structure in the aniline series. To the number of substances investigated by Staub should be added those studied by Halpern[26] and a great number described more recently by Viaud[27] in the laboratories of the Société Rhône-Poulenc, who has thoroughly investigated the chemical aspects of this problem.

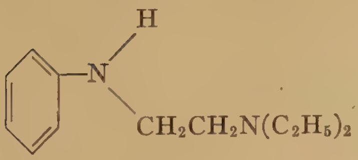
Starting with the basic structure common to both the molecule of 1571F and Antergan, it is a simple matter to follow the variations in activity brought about by molecular transformations.



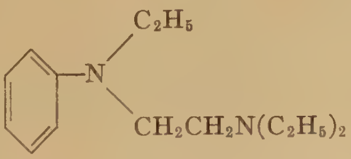
Protection: none



none



little

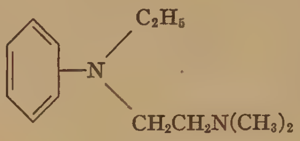


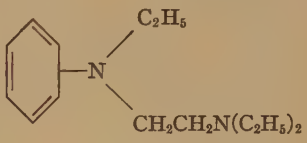
4 doses

The part played by substitutions on the amino groups, is demonstrated by the antihistamine effect of the four derivatives investigated in my laboratory, as shown in the previous diagram.

Antihistamine activity only appears when both amino groups are tertiary. Aminoethylaniline is inactive against histamine, although it is a hypertensor and a bronchodilator.

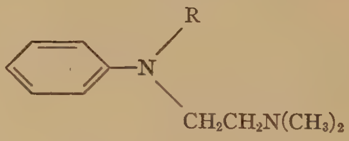
Besides the primary, secondary, or tertiary character of amines, the weight of the substituents introduced into the molecule predominantly influences the degree of antihistamine activity. It was shown that the substitution of dimethylamine for diethylamine in the side chain increased the activity of 1571 F. approximately two and one-half times.



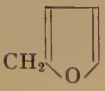
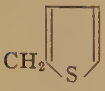
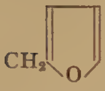
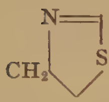


	2325 RP	1571 F
Protection secured against histamine shock (guinea-pig)	10 doses	4 doses
Protection against the toxic effects of a histamine aerosol (guinea-pig) mg/kg	5 mg	20 mg
Spasmolytic activity on the isolated intestine (2325 RP = 1)	1	¼

Of no less importance is the effect of substitutions made on the aromatic amino group. The activity, quite insignificant when R is substituted by

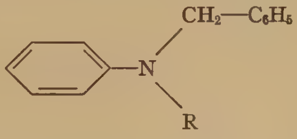


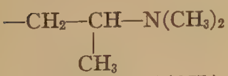
		Dose of antihistamine drug permitting to effect prevention of bronchospasm caused by inhalation of a histamine aerosol (mg./kg.)	Spasmolytic activity on the isolated intestine of guinea-pig (Antergan = 1)
(*a*)	H	no action	1/100
	CH_3	no action	1/100
	C_2H_5 (2325 RP)	5	1/15
	n C_3H_7	2.5	1/8
	n C_4H_9	2.5	1/4
	n C_5H_{11}	2.5	=
	n C_6H_{13}	8	1/10
	n C_7H_{16}	50	1/30

(*b*)	C_6H_5	50	=
	$CH_2 \cdot C_6H_5$ (Antergan)	1	1
	$CH.CH_2 \cdot C_6H_5$	no action	
(*c*)		1	1
		no action	
		20	1/20
		1	1/1.6

a simple methyl group, becomes greater as the molecular weight of the substituents is increased to an optimum with five carbon atoms. The maximum activity is reached with the benzyl radical. In addition to the hydrocarbon chains, many other groupings and nuclei have been introduced.

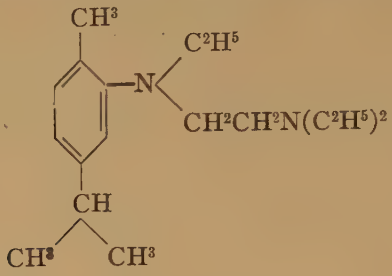
Moreover, the length of the aliphatic side chain appears to be a determining factor. Its role is similar to that which pharmacologists have observed



	Dose of antihistamine drug permitting to effect prevention of bronchospasm caused by inhalation of a histamine aerosol. (mg./kg.)	Spasmolytic activity on the isolated intestine of guinea-pig (Antergan = 1)
$—CH_2CH_2N(CH_3)_2$ (Antergan)	1 mg.	1
 $—CH_2—CH(CH_3)—N(CH_3)_2$	25 mg.	=
$—CH_2CH_2CH_2N(CH_3)_2$	20 mg.	1/15

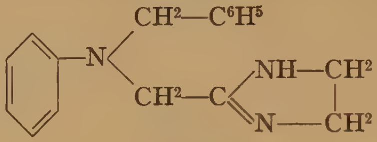
in the series of sympathomimetic and parasympathomimetic substances. The "optimum" is often represented by the β-amino-aliphatic chains, according to the rules laid down by Barger and Dale for adrenalin derivatives and by Hunt for cholines.

Lastly, let us mention the effect of substitutions made on the benzene nucleus. It is surprising to find that such alterations in the molecule



strongly interfere with the pharmacodynamic activity in the case of aniline derivatives, but have almost no effect in the phenolic ether series.

Antistine, or benzyl-N-methylimidazoline-aniline, belongs to the same chemical series which includes many active drugs such as Otrivine, Privine



Antistine

and Priscol[28] and was also prepared by Miescher, Urec, and Klarer. Its molecule is composed of a benzyl group linked to aniline. In the case of Antistine, however, the aliphatic amine of the Antergan molecule is replaced by a more complex base, namely the imidazoline nucleus. This substance has an antihistamine activity similar to that of Antergan. According to Meier and Bucher,[29] Antistine produces in guinea pigs the typical effects of the antihistamine drugs, but higher doses are necessary to counteract histamine intoxication. In our tests, the ratio between Antergan and Antistine activity was found to be about 10 to 1.

With the group of phenolic ethers and the group of aniline derivatives in which the oxygen bridge of the phenolic ethers has been converted into a nitrogen bridge, we have briefly examined the part played by the amino substituents, by the substitutions of the benzene nucleus, and by the transformations effected in the side chain. There remained for consideration the influence on antihistamine activity of the replacement of the benzene nucleus itself by other radicals. Toward this end, we investigated in 1944, together with Walthert, Fournel, and Horclois,[30, 31] the derivatives of aminopyridine and described the activity of the most active compound, namely, N-p-methoxybenzyl-N-dimethylaminoethyl-α-aminopyridine, or Neoantergan. Neoantergan is the most active of all hitherto known antihistamine agents. By subcutaneous injection it exerts a preventive action against histamine intoxication in doses of less than one-tenth of a milligram (FIGURE 2).

The experiments which led to the discovery of Neoantergan included more than 30 derivatives belonging to the same chemical series. This was reported upon in detail by Viaud[27] in 1946 and by me in 1947 at the International Congress for Chemistry in London.[32] In 1945, Mayer, Huttrer, and Scholz,[33] working in the same direction, recognized the activity of N-benzyl-N-dimethylethyl-α-amino-pyridine, which they introduced under the name of Pyribenzamine.[34] They have established the first elements of the rules which in this group govern all relationships between pharmadynamic activity and chemical structure. Pyribenzamine is about five times less active than Neoantergan in animals.

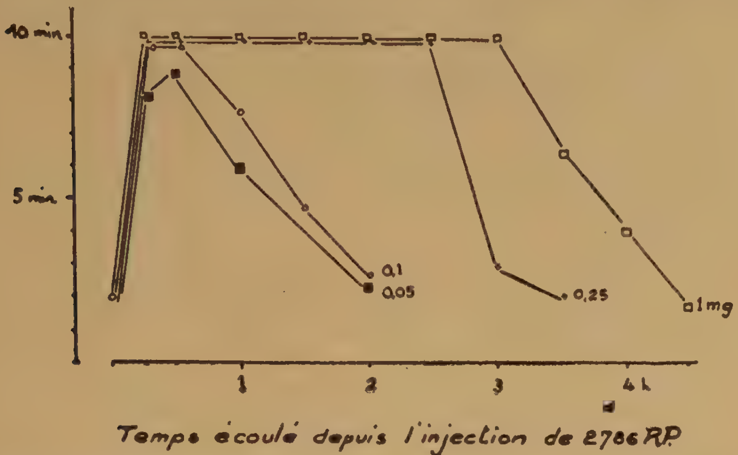
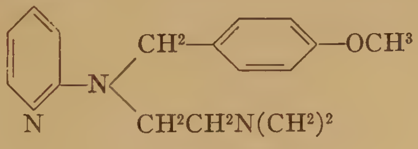
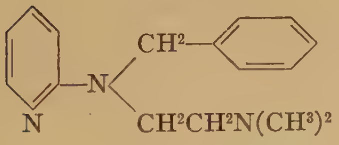


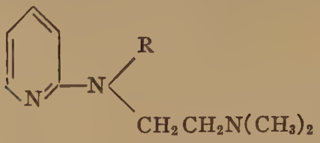
FIGURE 2. Antihistaminic activity of Neoantergan. Protection against bronchospasm induced in guinea pigs by inhalation of a histamine aerosol. Effects of increasing doses of Neoantergan injected subcutaneously into guinea pigs (mg./kg.). Ordinate: time of animal exposure in the inhalation box, before the appearance of severe asthma; Abcissa: time between injection of Neoantergan, and exposure to aerosol. The untreated animals show an average resistance of 2 minutes against the effect of histamine. Resistance is prolonged by a preventive injection of a single dose of Neoantergan (injected in time 0) (Bovet and Walthert[31]).

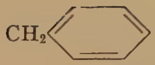
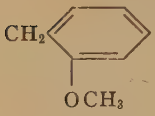
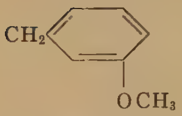
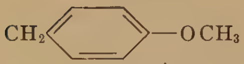
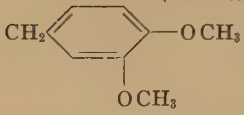


Neoantergan



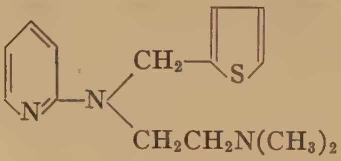
Pyribenzamine



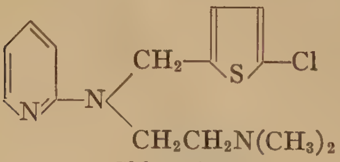
	Dose of antihistamine drug permitting to effect prevention of bronchospasm caused by inhalation of a histamine aerosol (mg./kg.)	Spasmolytic activity on the isolated intestine of guinea-pig (Antergan = 1)
 (Pyribenzamine)	0.5	1.3
	10	1/20
	no action	=
 (Neoantergan)	0.1	1
	no action	1/80

If the methoxy group of the Neoantergan molecule is transferred from the paraposition of the benzene nucleus to the meta- or ortho-position, the activity of the compound decreases considerably.

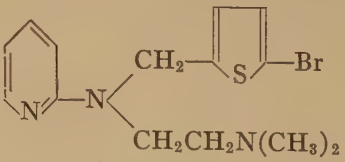
The thiazole derivatives of these aminopyridine compounds have been introduced[35, 36] under the name of Thenilene or Histadil, and the corresponding chloro- or bromoderivatives under the name of Chlorothen and Bromothen respectively.[36]



Thenilene



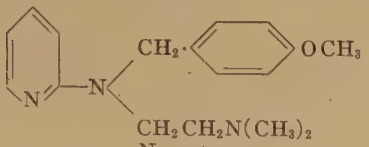
Chlorothen



Bromothen

It is somewhat surprising that the physiological properties of the pyridine derivatives vary, to a marked extent, with certain substitutions which do not produce the same variations in the case of the aniline derivatives of the Antergan group. Thus, the position of the amine in the pyridine nucleus plays an important role.

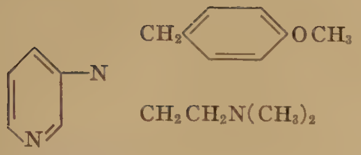
(*a*)



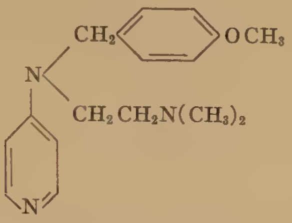
Neoantergan

(2786 RP)

(*b*)



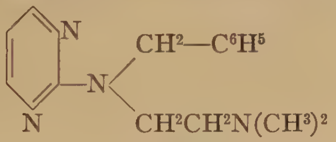
(*c*)



Dose of antihistamine drug permitting to obtain prevention of bronchospasm caused by inhalation of a histamine aerosol:

(*a*) 0, 1 mg. (*b*) 10 mg. (*c*) 50 mg.

Feinstone, Williams, and Rubin[37] have recently described the antihistaminic properties of a pyrimidine derivative, Hetramine. By comparison



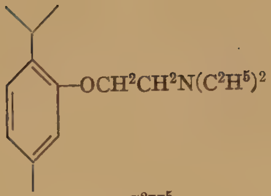
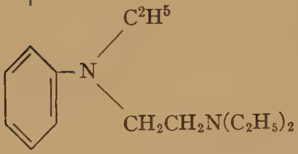
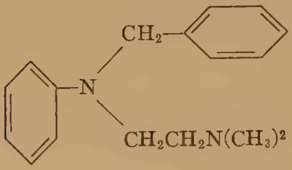
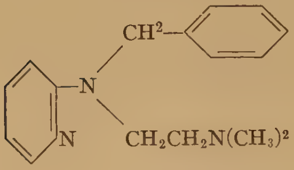
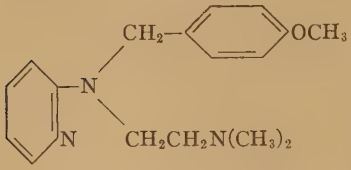
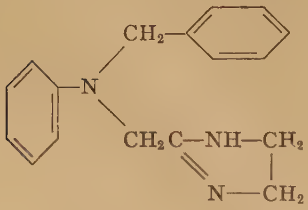
Hetramine

with the antihistaminic substances already mentioned, this newcomer possesses only a low activity.

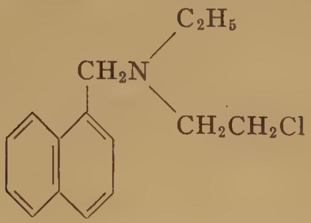
From the standpoint of their antihistamine properties, each of the various chemical series we have considered so far, namely, phenolic ethers, anilines, aminopyridines, have contributed toward definite progress. The protection afforded against histamine approached first 5, then 120, and later 300 lethal doses. The minimum active dose of antihistamine agent decreased from 20 mg. to 1 mg. and finally to 0.1 mg.

It is important to emphasize that Neoantergan is, like histamine, a triamine. This is possibly more than a mere coincidence and may indicate that we are dealing with a competitive phenomenon at the level of peripheral receptors.

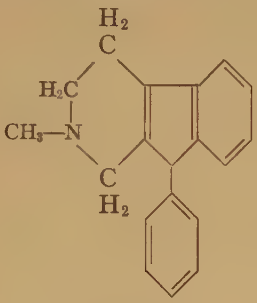
From the chemical standpoint, the four series of compounds already mentioned, namely, phenolic ethers, aminoethylanilines, phenylaminomethylimidazolines, and aminopyridines, have certain structural relationships to

		Toxicity mouse s.c.mg/kg	*Protection against histamine shock*		*Protection against the toxic effect of a histamine aerosol*		*Protec. against anaphylactic shock*
			number of doses	*minimal active doses*	*mg/kg*	*duration 1 mg/kg 10 mg/kg*	*mg/kg*
	929 F	500	5 d.		20 mg.		
	1571 F	750	10		10 mg.	10 min.	
	Antergan (2339 RP)	175	120		1	2h.	1 mg.
	Pyribenzamine	100	150	0.005	0.5	2h.	1 mg.
	Neoantergan (2786 RP)	150	300	0.0015	0.1	3–4h.	0.2 mg.
	Antistine	150	30		10	1h	2–3 mg.

different groups of known sympathomimetic and sympatholytic compounds. One could add to this list, two other series, namely, alkylaromatic derivatives of β-chlorethylamine, recently described by Achenbach and Loew,[38]



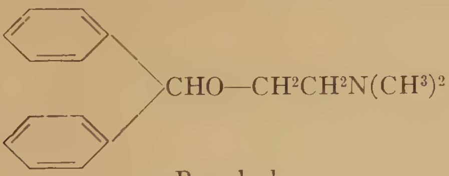
and phenyl tetrahydro-pyridindenes, the activity of which has been reported by Lehmann, Hagen, Berberow, and Roe.[39]



Thephorin

Two other groups not hitherto mentioned in this discussion, but which we shall now describe in some detail, are quite different from all previous groups in so far as they no longer are associated with any sympathetic activity. Rather, they are related to the spasmolytic agents and the acetylcholine antagonists. These groups are benzohydrylethers and phenothiazylethylamines.

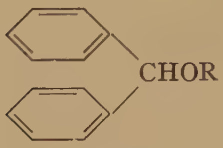
Indeed, considering their structure, the ethers of benzyl alcohol, prepared by Rieveschl and Huber and investigated by Loew, Kaiser, and Moore,[40–42] are more closely connected with the spasmolytic agents of the Trasentine type than all preceding series. Benzohydrylethers, the most active of which is Benadryl, represent a very original contribution of American scientists to the antihistamine problem. The minimal active dose of Benadryl for the guinea pig is 5 mg. when given under the experimental conditions of



Benadryl

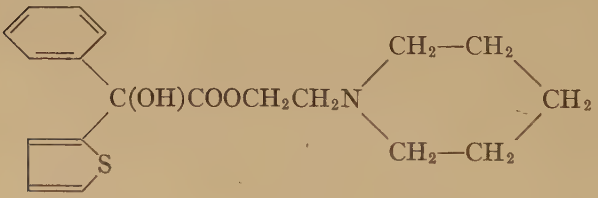
our own laboratories, whereas Loew found a threshold of activity of 1.5 mg. He has also found, however, for our products, a higher activity than that which we had established ourselves. Benadryl affords protection for the guinea pig against 75 toxic doses of histamine.

Loew and his co-workers have published some precise data concerning the relationship between the molecular structure and antihistamine activity in the Benadryl series. Several conclusions may be drawn from Loew's investigations, similar to those which we have formulated with regard to phenolic ethers. The most active side chain has two carbon atoms; tertiary amines have a higher activity than secondary or primary amines; and the dimethyl derivative is more active than the diethyl derivative. Loew[41] has also shown the high activity of the iodomethylate derivative of Benadryl. Other derivatives of the Trasentine group have been found active by Chen and Abreu,[43] such as:

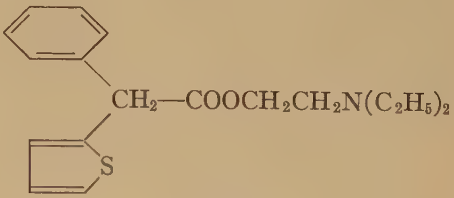


	Protection of the guinea pig against the toxic effects of histamine aerosol. mg./kg.
R = $CH^2CH^2NH^2$	25 mg.
$CH^2CH^2NHCH^3$	6 "
$CH^2CH^2N(CH^3)^2$	1.5 "
$CH^2CH^2N(C^2H^5)$	12.5 "
$CH^2CH^2NC^5H^{10}$	1.5 "

(according to Loew, Kaiser and Moore, 1945)



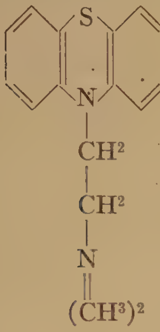
PPT



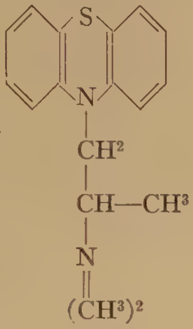
DPT

We shall terminate this long list of active compounds by referring to the phenothiazines, the study of which we had begun at a time when we were engaged in a search not for antihistaminics, but for antimalarials. We are still investigating this group in our laboratory. The first results on the antihistaminic activity of these substances have been reported by Halpern.[44] The first synthesis of these phenothiazines was described in this country by Gilman,[45] but no pharmacologic investigation had been reported.

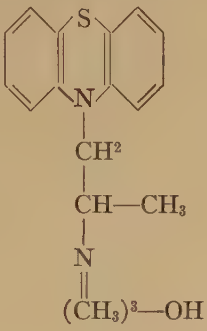
Although their threshold of activity is higher than that of Neoantergan, they are of special interest because of their long-lasting and powerful action. Phenothiazinylethyldimethylamine (3015 RP) is active in a dose of 0.5 mg. and protects the guinea pig against 700 lethal doses of histamine. A corresponding isopropyl derivative, namely, 3255 RP, is still more active.



3015 RP

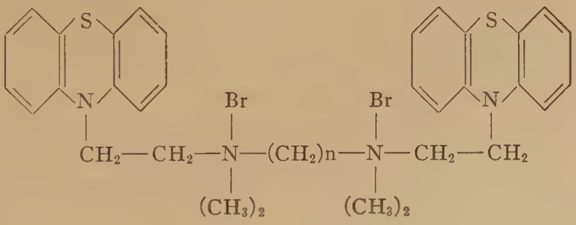


3277 RP

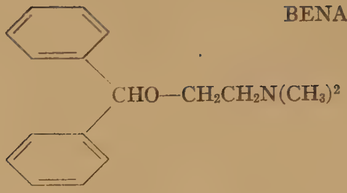
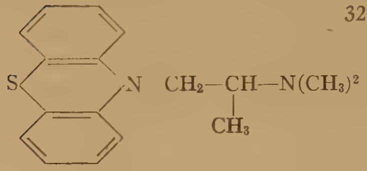
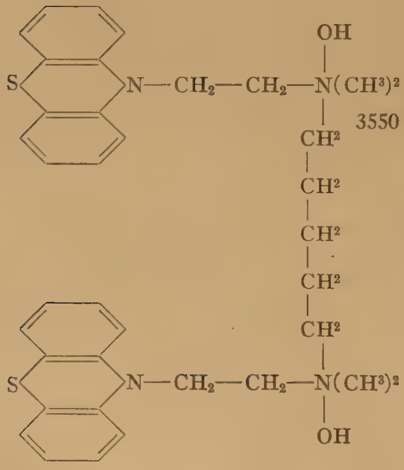


3554 RP

We are now investigating certain derivatives of the same group with a quaternary function and various other derivatives of high molecular weight which are better tolerated.[32]



	Protection of the guinea-pig against the toxic effect of a histamine aerosol		Protection secured against histamine shock
	mg./kg.		number of doses
n = 2	0.5	2-3 h.	400
n = 4	0.5	6 h.	500
n = 5 (3550 RP)	0.5	6 h.	700
n = 6	1.0	5 h.	600
n = 7	1.0	5 h.	400

	Toxicity mouse sc. mg./kg.	*Protection against histamine shock*		*Protection against the toxic effect of a histamine aerosol*		*Protec. against anaphyactic shock*
		number of doses	*minimal active doses*	*mg./kg.*	*duration mg. 10mg.*	*mg. kg.*
BENADRYL 	150	75	0.06	5	—3h	1–2mg.
3277 RP 	225	1200	0.02	0.5	7h —	0.1mg.
3550 RP 	500	700	—	0.5	6h —	

Having thus reached the end of this enumeration, we shall now attempt to establish the characters common to the various series of antihistamine products. Actually, only a few functions are common to these different groups. In the first place, they all possess one or more strongly saturated tertiary or quaternary amines. Secondly, the amino group is frequently found in β position, attached to an aliphatic chain. Thirdly, an amino group or an oxygen atom forms a linkage between a strongly electropositive nucleus and a basic side chain.

In addition to these very general rules, there exists in each of the series considered a characteristic element which emphasizes its activity. The role of these elements still escapes us entirely, so that all investigations in this field have a highly empirical character. In the series of phenolic ethers, substituents on the benzene nucleus play a part which does not appear to have any significance in the case of the aniline compounds. Indeed, the OCH_3 group has a great importance when attached to the benzyl group of Neoantergan but is of no importance if present in the benzyl group of Antergan. In the phenothiazine series, the branching of the side chain plays an important role, which is lacking in the other series. We want to stress once again the significant fact that antihistaminic activity could always be detected in those substances which are structurally related to adrenolytic or spasmolytic compounds.

In a general way, this chemical study leads to the conclusion that only in one instance have we been able to establish a certain structural relationship between the antihistaminic agents and histamine itself.

In spite of the chemical diversity of the various antihistaminic agents, there exists a great homogenicity in their pharmacodynamic properties. TABLE 1 illustrates the general activity of the various antihistaminics upon histamine effects.

I shall now report some quantitative data relevant to Neoantergan, which, as has been shown, is a particularly active product. The concentrations active on isolated organs are often smaller than those of histamine itself. Dews and Graham[46] have reported that, on the rabbit ear, which is particularly sensitive, this product exerts an activity in a dose of 1 thousandth of a microgram. By subcutaneous injection, Neoantergan manifests, in the intact animal, an antitoxic action in a dose of .03 mg. per kg. According to Winter,[47] the ratio for Neoantergan between the active dose in guinea pigs treated with a normally toxic dose of histamine and the LD_{50} is 1 to 68,000.

The protection against one or several lethal guinea-pig doses of histamine represents the most specific criterion so far employed. The definitely toxic dose of histamine for an unprotected animal injected intrajugularly varies between 0.4 and 0.9 mg./kg. The test method consists of injecting increasing doses of histamine successively into the vein of the guinea pig, protected by the subcutaneous injection of the drug. The results are given in a previous paper,[9] with reference to the toxic dose of histamine for normal animals.

As already indicated, this method has been subsequently utilized by Staub[10] (phenol and aniline derivatives); by Rosenthal and Brown[13] (929 F); by Burchell and Varco[16] (929 F and 1571 F); and by Halpern[26] (Antergan). Remarkable results have been obtained with Neoantergan, whose activity is noticeable at the (subcutaneous) dose of 0.1 mg./kg.[30, 31] (see TABLE 2).

The maximum protection assured by the compounds belonging to the different chemical series have been mentioned previously. Friedlaender, Feinberg, and Feinberg[48] have expressed the antihistaminic activity by the number of doses of histamine necessary to cause death in the guinea pig

TABLE 1

ACTION OF THE ANTIHISTAMINICS ON THE PHARMACODYNAMIC EFFECTS OF HISTAMINE

Toxicity		
Guinea pig		protection (T, A, N, B, P,)*
Rabbit		protection (N)
Dog		protection (N)
Mouse		no protection (N, P,)
Arterial pressure		
Rabbit	hypertension	antagonism (T, A, N,)
	hypotension	weak antagonism (T, A, N,) or without action (T, A, N,)
Dog	hypotension	antagonism (T, A, N, B, P,)
Cat	hypotension	weak antagonism (N) unstable action (P)
Vasomotor effects		
Rabbit		
isolated ear	vasoconstriction	antagonsim (N)
coronary	vasoconstriction	antagonism (N)
Dog		
-plethysmograph of the nasal cavity	vasodilatation	antagonism (N)
-spleen	vasodilatation	antagonism (N)
-hind foot	vasodilatation	antagonism (N)
-coronary	vasodilatation	antagonism (N)
Cat		
-spleen	vasodilatation	antagonism (N)
-coronary	vasodilatation	antagonism (N)
Man		
-skin	vasodilatation	antagonism (A, N, B, P,)
Cardiotonic effect		
Rabbit		
isolated auricle	augmentation of the degree of contractions	antagonism (N)
Effects on the capillary permeability		
Dog	augmentation of the permeability	antagonism (N, B,)
Man	Lewis's triple reaction	antagonism (A, N, B, P,)
Dog	hemoconcentration	antagonism (T)
Effect on the bronchial muscles		
Guinea pig		
isolated lung	bronchoconstriction	antagonsim (T, A, P)
lung *in situ*	bronchoconstriction	antagonism (T, A, N)
Rabbit	bronchoconstriction	antagonism (T, N)
Dog	bronchoconstriction	antagonism (A, N, P)
Effect on the stomach muscles		
Guinea pig	contraction	antagonism (T)
Effect on the intestine muscles		
Guinea pig	contraction	antagonism (T, A, N, B, P)
Rabbit	contraction	antagonism (A)
Dog	contraction	antagonism (T, N, B, P)
Effect on the uterus muscles		
Rat	contraction	without effect (A)
Guinea pig	contraction	no antagonism or weak antagonism (T, A)
Rabbit	contraction	no antagonism or weak antagonism (A, N)
Dog	contraction	antagonism (N, B, P)
Lachrymal secretion		
Cat	Hypersecretion	antagonism (P)
Salivary secretion		
Cat	Hypertension	antagonism (P)
Gastric secretion		
Pigeon	Hypersecretion	without effect (A)
Rat	Hypersecretion	without effect (N)
Dog	Hypersecretion	without effect, exceptional antagonism (T, A, B)
Man	Hypersecretion	without effect or unconstant action (A, N, B, P)

*T = Thymoxyethyldiethylamine (929 F.); A = Antergan; N = Neoantergan; B = Benadryl; P = Pyribenzamine.

previously protected by the subcutaneous injection of 3 mg./kg. of the drug. For the different products, they have found the following results:

	Antihistaminic activity[48]
Benadryl	5
Antergan	6
Pyribenzamine	37
Neoantergan	125

With the strong dose of 20 mg./kg. of a derivative of thiodiphenylamine (3277 RP), one can succeed in partially protecting guinea pigs against 1200 toxic doses of histamine. However, animals die eventually from gastric ulcers and perforations.[44]

Winter[47] has developed a somewhat different test, which permits the determination of the dose capable of protecting 50 per cent of the animals.

TABLE 2
ANTITOXIC ACTIVITY OF NEOANTERGAN AND ANTERGAN[31]

	Dose injected subcutaneously—mg/kg.	*Number of treated animals*	*Intravenous histamine*	
			number of minimal lethal doses tolerated	*total quantity of histamine received per kg. in one injection (mg.)*
Neoantergan	0.1	4	5 to 10	3 to 6
	0.25	2	15 to 20	9 to 12
	0.5	4	30	18
	1	11	75	45
	20	—	300	180
Antergan	1	2	3 to 5	1.8 to 3
	2.5	5	30	18
	5	5	40	24
	10	6	45	27
	20	—	120	72

In this way, considering the toxicity of the products for the mouse, Winter established a therapeutic index which appears to be very high for some derivatives (TABLE 3).

The effects caused by histamine in different species of animals cannot be superimposed. While spasmodic phenomena predominate in the guinea pig, vasomotor phenomena are relatively more important in the dog. Moreover, the lethal doses of histamine vary on a large scale from one species to another. By intravenous injection, nearly 0.5 mg./kg. of histamine is sufficient to kill the guinea pig, 2 to 20 mg./kg. will kill the rabbit and the dog, while 400 mg./kg. are required to kill the mouse.

As with these differences in the susceptibility to histamine, there also exist considerable differences in the reactions provoked by the antihistamine substances in various animal species. In a general way, the greater the susceptibility to histamine, the higher is the protection.

We have repeated on the rabbit and on the dog the same assays as performed on the guinea pig.[31] For the rabbit, the protection assured by Neoantergan approaches 30 minimal lethal histamine doses; in dogs, the protection extends only to two and a half toxic histamine doses. In mice, it has been shown that not only did the antihistaminics confer no protection, but the toxicity of these antihistaminics was added to that of histamine. This phenomenon, formerly described for Neoantergan,[31] has been confirmed during the study of Pyribenzamine.[49]

TABLE 3
ANTITOXIC DOSE, THERAPEUTIC INDEX, AND TOXICITY PECULIAR TO CERTAIN ANTIHISTAMINIC DRUGS*

	Antihistaminic effect—minimal active dose	*Toxic dose D.L. mouse (i.p.)*	*Therapeutic index*
Hetramine	0.150	76	500
Benadryl	0.060	140	2,333
3277 R.P.	0.020	190	9,500
Pyribenzamine	0.003–0.005	68	19,400
Neoantergan	0.001–0.005	102	68,000

* Data taken from Winter.[47]

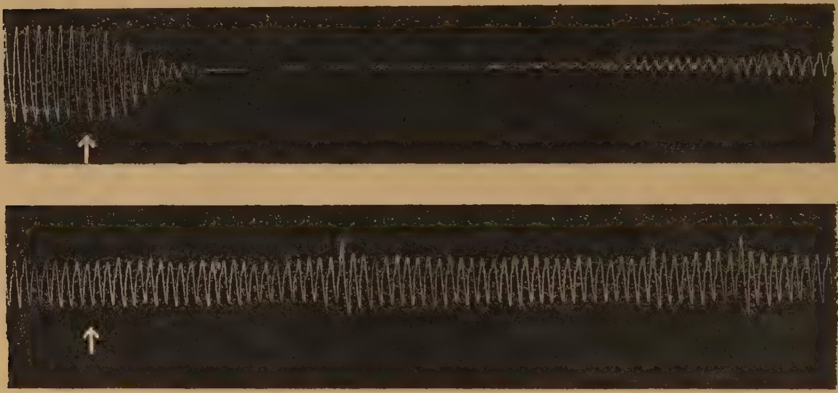


FIGURE 3. Action of thymoxyethyldiethylamine (929 F) on the histaminic bronchoconstriction of the guinea pig. Registration of the bronchial caliber *in situ* according to the technique of Cordier and Magne. *1st line:* action of 0.05 mg./kg. of histamine hydrochloride (i.v.). *2nd line:* action of the same dose of histamine in an animal previously treated with 5 mg./kg. of 929 F. intravenously (Staub[10]).

The protection of guinea pigs against one or more lethal doses of histamine and the prevention of histaminic asthma by antihistaminics given in the form of an "aerosol" represent a uniform and constant phenomenon, namely the suppression of bronchial spasm provoked by histamine. Experimentally, it may be observed by different methods.

According to the method of Cordier and Magne, which we used in our early work with Staub,[10] the guinea pig is placed in a closed box and air is introduced under a regular pressure directly into the trachea. The recording of the velocity of the air passing through the trachea after every inspiration, or of the quantity of air penetrating the thoracic cavity, proves that histamine provokes an intense bronchoconstriction which does not occur after the animal has been treated with thymoxyethyldiethylamine (929F) (FIGURE 3).

According to a technique of Konzett and Roessler, utilized by Halpern[26] in his study on Antergan, the volume of air introduced is constant, and the portion of the air which does not penetrate into the lungs in great quantities is recorded after every inspiration in a manometer. We have verified once again, with Walthert,[31] the utmost sensitivity of this method. Following the injection of 1 mg. of Neoantergan into the jugular vein of the guinea pig, the sensitivity of the guinea pig towards histamine is diminished by 50 per cent. After 1 mg./kg., a dose of histamine 30 times greater than the normal dose is needed to produce bronchoconstriction (FIGURE 4).

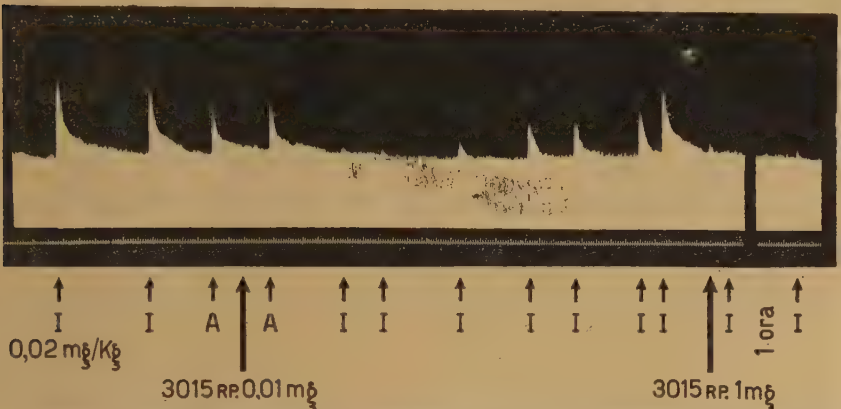


FIGURE 4. Activity of small doses of the antihistaminic drug, 3015 RP (phenylthiazinilethyldimethylamine), on the bronchospasm produced by intravenous injection of histamine in the guinea pig. The diminution of the bronchial caliber is characterized in this experiment (using the technique of Konzett and Roessler) by an increase of the height of the graph. It may be noticed that 3015 RP counteracts the bronchoconstricting effects of histamine (I), whereas it does not alter in any way the bronchoconstriction induced by acetylcholine (A). A small dose of 3015 (0.01 mg./kg.) produces transitory effects; the action is prolonged after a larger dose (1 mg.) (Bovet 1947[1]).

Using the plethysmography of the lung, as described by Jackson, Yonkman[104] has observed the antagonistic effect of Pyribenzamine on histaminic bronchoconstriction in the dog. With the isolated lung of the guinea pig, prepared according to the method of Sollmann, Staub has been able to demonstrate the antagonism towards histamine of the products, 929F and 1571 F,[11] and Yonkman that of Pyribenzamine,[50] thus demonstrating the peripheric nature of the protective effect.

Besides the bronchia, many other smooth muscular fibers of the intestines, stomach, uterus, and bladder respond with a change of tonus to the administration of histamine, usually presenting intense spasms to the administration of very minute doses. This is particularly evident with the intestinal muscles of the guinea pig, which react to a histamine concentration of the order of one to a billion.

The use of the isolated guinea-pig intestine constitutes the preferred criterion for the demonstration of the effectiveness of various antihistaminic substances. They prevent the histamine spasm or bring about the relaxation of the contracted intestine. The effect of histamine at the concentration of 10^{-7} (0.1 mg./1) on the guinea-pig gut is inhibited by the same concentration of 929 F or 1571F.[9, 11] With Antergan,[26] Neoantergan,[31, 51] and Pyribenzamine,[33, 47] the inhibiting dose is frequently smaller than the dose of histamine producing contraction (FIGURE 5).

On the other hand, the study of spasmolytic effects of the different compounds is excellently suited for the determination of the specificity of their properties. Antihistaminics oppose histaminic hypotension. This action was described for the first time by Rosenthal and Minard[12] while they were investigating thymoxyethyldiethylamine. The hypotension provoked in ten dogs by an intravenous injection of 4 mg. of histamine averaged 40 mm. at the beginning of the experiment, while it was of no more than 10 mm. after administration of 929F. Observations of the same kind, but more demonstrative, have been made with Antergan,[50] Neoantergan,[31, 52] Pyribenzamine,[50, 52] and Benadryl[41, 52, 53] (FIGURES 6 and 7).

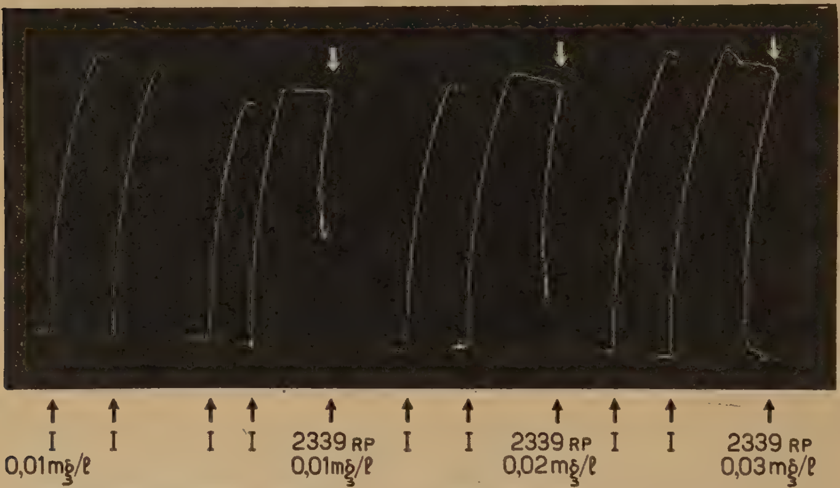


FIGURE 5. Action of Antergan (2339 R.P.) on the histaminic spasm of the isolated intestine of the guinea pig (I) (Bovet, 1947[1]).

According to the observations of Sherrod,[52] Neoantergan, Pyribenzamine, and Benadryl have the same activity on the arterial pressure of the dog. Wells, Morris, Bull, and Dragstedt[53] have shown that the hypotensive effects produced in the dog by doses of 0.001–0.002 mg. of histamine are abolished by a dose of 3 mg./kg. of Benadryl. Examination of the responses indicate that a competitive antagonism similar to that between atropine and acetylcholine exists between Benadryl and histamine. The antagonism exerted by the antihistamine substances towards the tensional effects of histamine on the dog is impressive, although not so intense and long-lasting

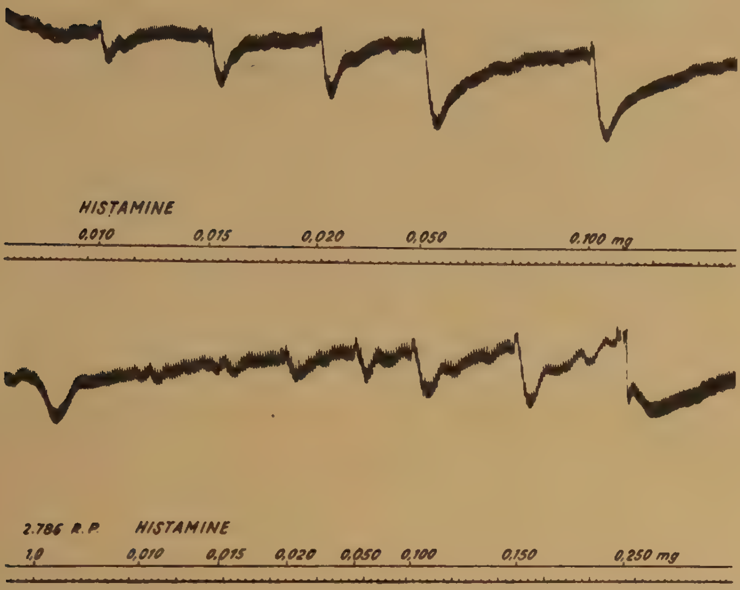


FIGURE 6. Antihistamine activity of Neoantergan. In a dog treated with 1 mg. of Neoantergan (2nd line), histamine has only slight tensional effects on the blood pressure (narcosis with chloralose; registration of arterial pressure; and intravenous injections of histamine chlorydrate). Doses in mg./kg.; time: ten seconds (Bovet and Bovet-Nitti, 1948[1])

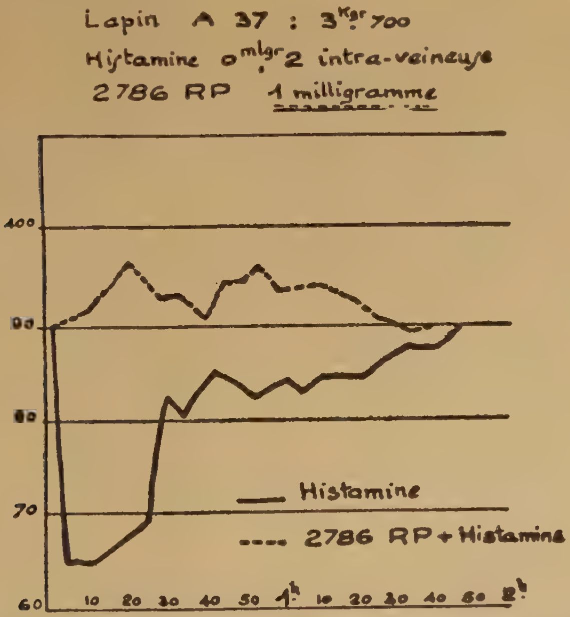


FIGURE 7. Effect of Neoantergan on the histaminic hypotension in the rabbit. Rabbit unanesthesized registratio nof the arterial tension according to Grant and Rothschild. The same animal served in the two tests performed with an interval of a week. The intravenous injection of 0.2 mg./kg. of histamine causes a fall of blood pressure in the normal animal (—) while, in the animal protected by a subcutaneous injection of 1 mg./kg. of Neoantergan (2786 RP) given a quarter of an hour previously (.....), it remains without effect (Ramanamanjari, 1944, cit.[31]).

as that which one may observe, for instance, on the bronchia of the guinea pig.

It appears, from these investigations, that poisons of this type oppose not only the motoric effects but also the inhibiting effects of histamine. In this regard, the data on the antagonistic effects on the vasomotors of the intestines of various species are particularly instructive. We must mention here particularly the observation of Dews and Graham[46] on Neoantergan. They report that Neoantergan antagonizes not only the vasoconstricting effects produced by histamine on the blood vessels of the rabbit ear but also the vasodilatation effect in the paw of the dog and of the coronary arteries of the cat and the dog. Similar observations have been made on histaminic vasoconstriction of the coronaries of the rabbit,[54] and on vasodilatation of the nasal cavity[31] and splenic vasoconstriction in intact animals.[46]

Meier[29] has also noted that the vasoconstriction caused by perfusion of the paw of the rabbit with histamine in a relation of 1/1,000,000 instantly disappeared under the effect of Antistine in a dilution of 1/1,000,000. Neoantergan also opposes the tonic effect produced by histamine on the amplitude of the contractions of the isolated auricle of the rabbit.[46]

According to the tensional and vasomotor effects just mentioned, histamine produces, at the same time, a lesion of the capillaries characterized by an increase of their permeability. This process leads to a diffusion of plasma, leading to a diminution of the blood volume as indicated by an increase in the number of blood cells. In this way, histamine accelerates and facilitates the diffusion of the coloring substances introduced into the circulation and their fixation in the skin. The vasodilatation and the damage to the capillaries with their consequences, hypotension and hemoconcentration, constitute an acute phase of histamine intoxication.

Researches on the protection exerted by the antihistaminics in this field are numerous. Celice and Durel[55] and Parrot[56] have stated that, in man, the local susceptibility to histamine (Lewis's triple reaction) is diminished or suppressed by Antergan, and their findings have been frequently confirmed[57] and applied to other products such as Neoantergan,[46] Pyribenzamine,[58] and Benadryl.[59]

Antihistaminics do not oppose, in most cases, the gastric secretion produced by histamine. Any effect is usually secondary and probably due to the atropinic action the antihistaminics exert. The first observations on this subject have been made by the investigators who studied thymoxyethyldiethylamine (929F) and N-diethyl-aminoethyl-N-ethylaniline (1571F) on the dog: Loew and Chickering,[17] Burchell and Varco,[16] Bourque,[18] and Hallenbeck.[19] They noted that the inhibition of the gastric secretion, when it occurred, was not specific, since these substances also depressed the hypersecretion caused by pilocarpine[18] or mecholyl[19] in the same way they suppressed histaminic hypersecretion. In a general way, the antagonism appeared to be more obvious on the physiological response of the gastric secretion to food than to histamine.[19]

Similarly, Antergan does not modify the character of the gastric secretion. This has been verified by Gordonoff[60] on the dog and on the pigeon.

Also, Neoantergan does not modify the gastric secretion in the rat following the injection of histamine[31] (FIGURE 8). The newer antihistaminics, although much more active against histamine, have no more consistent effect on the gastric secretion. The results with Benadryl[59, 62] and Antergan[63] are still irregular, in both the laboratory[41, 61] and the clinic.

The study of the antihistaminic agents has furnished interesting observations on the physiological function and physiopathologic effects of histamine. From the beginning of our studies, we were impressed by the absence of any specific activity of the antihistaminic substances on the normal organism.

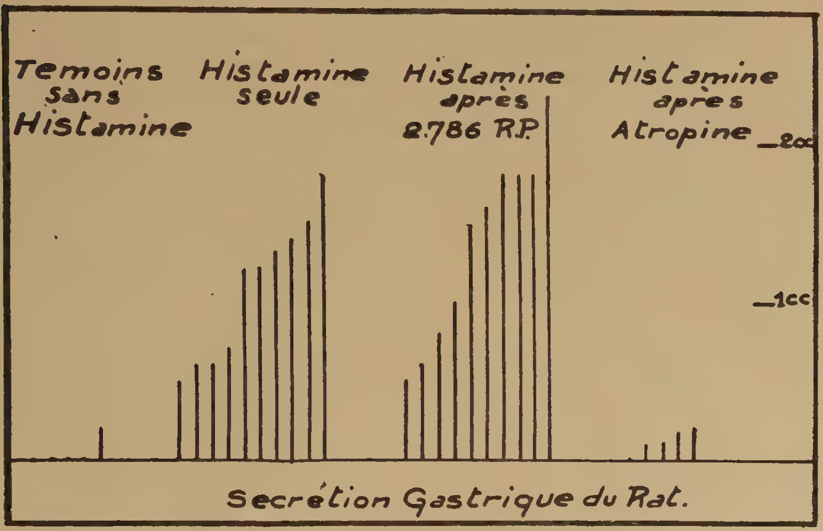


FIGURE 8. Neoantergan does not counteract the gastric hypersecretion produced by histamine in the rat, while atropine inhibits it. Tests on the rat: histamine 50 mg./kg. subcutaneously; Neoantergan 50 mg./kg. subcutaneously; atropine 200 mg./kg. subcutaneously. Each column corresponds to the quantity of gastric juice present in the stomach of the rat 1½ hours after the administration of the histamine. Results: (1) increase in the gastric secretion after histamine; (2) the preventive administration of Neoantergan (2786 RP) 30 minutes before histamine administration did not prevent histamine hypersecretion; (3) atropine, injected under the same conditions, prevented almost entirely the histaminic gastric hypersecretion (Bovet and Walthert[31]).

In normal animals, atropine produces characteristic symptoms which must be interpreted as nullification of normal acetylcholine functions. The fact that Neoantergan does not produce any specific untoward effect in normal animals could be interpreted as a proof that histamine has no important physiological function. The few minor disturbances following injection of Neoantergan into a healthy individual are difficult to explain on the basis of a counteraction of important histamine functions. It is possible, however, to save the life of a guinea pig after administration of a lethal dose of Neoantergan by an injection of histamine.[54] This fact demonstrates, for the first time perhaps, that, to use a familiar expression, histamine "serves for something."

Experiments made with synthetic antihistamine drugs constitute, in both the laboratory and the clinic, a striking demonstration of the hypothesis that histamine represents a predominating factor in causing anaphylactic and allergic troubles. The results of different authors on the antihistamine substances indicate a close parallelism between the antihistaminic properties and the antianaphylactic properties of synthetic derivatives. The use of antihistamine drugs permits us, today, to establish the boundaries of that large domain of allergy which depends in a particular way on the reactions provoked by histamine.

The antiallergic activity of the antihistamine substances manifests itself distinctly during the following syndromes:

(*a*) anaphylactic shock of the guinea pig;

(*b*) anaphylactic shock of the rabbit;

(*c*) anaphylactic shock of the dog with humoral, cardiovascular, digestive, respiratory, and nervous involvement;

(*d*) Schultz-Dale reaction, provoked by the addition of allergen to the isolated organs of a sensitized animal;

(*e*) cutaneous allergy;

(*f*) allergic reactions to chemical agents;

(*g*) Sanarelli-Shwartzman reaction of local sensitization; and

(*h*) experimental nephritis of Matsugi (inverted allergy).

The first observations on the treatment of anaphylactic conditions with antihistamine drugs have been made on the guinea pig, the ideal animal for this purpose. Animals sensitized with one or more subcutaneous or intraperitoneal injections of heterogeneous albumin (ovalbumin, serum) are, after three weeks, abnormally sensitive to the intravenous injection of a minute dose of protein and present the Arthus phenomenon.

With Staub,[11] we have noted that the administration of 929F (40 mg./kg. s.c.), thirty minutes before the re-injection of appreciable amounts of serum, exerts a protective action. This observation has been repeated several times (FIGURE 9). The type of antigen and the method of administration have been changed, but the results are of the same order, protection being afforded in a regular manner.

Rosenthal and Brown's experiments[13] were on guinea pigs sensitized three weeks prior to treatment with an intraperitoneal injection of 20 mg. of ovalbumin. They showed that, in untreated animals, a dose of 0.04 cc. of antigen provoked anaphylactic shock, while animals protected by 929F (40 mg./kg.) presented no reaction, even after receiving a 2.5 times greater dose of antigen.

We were also able to obtain protection with 0.1 mg./kg. of Neoantergan[30, 31] when the shocking dose was administered by inhalation of an aerosol of ovalbumin. During one experiment, an inhalation of aerosol containing 10 per cent ovalbumin was administered to several young sensitized guinea pigs. In unprotected animals, severe and generally irreversible reactions appeared rapidly, while all treated animals survived and showed, if any, only moderate anaphylactic symptoms (TABLE 4).

Several antihistaminic drugs have been employed in this test. Staub,[11] Wilcox and Seegal,[25] and Loew[64] have described the activity of 1571F; Halpern[26] has found that Antergan protects in a dose of 20 mg./kg., which seems to be greater than the therapeutic dose; Loew, Kaiser, and Anderson,[65] Friedlaender, Feinberg, and Feinberg,[66] and Selle[67] have recognized the action of Benadryl; Mayer, Huttrer, and Scholz,[33] Baer and Sulzberger,[68] and Friedlaender[66] that of Pyribenzamine; and similar properties have

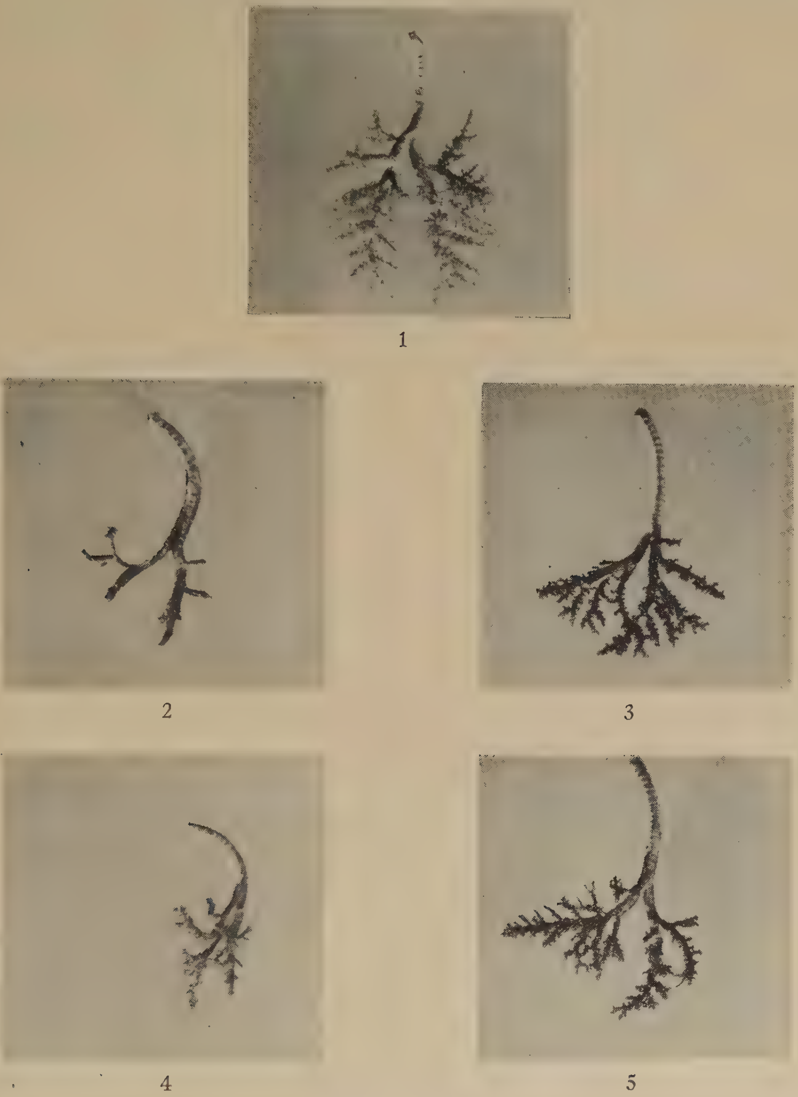


FIGURE 9. Celloidin casts of guinea-pig lungs according to Hanzlick. (1) Control, normal lung. (2) Lung of a guinea pig intoxicated by one lethal dose of histamine (1 mg./kg.); incomplete filling due to bronchial obstruction. (3) Protection given by an injection of Neoantergan to the guinea pig intoxicated by the same dose of histamine. (4) Incomplete filling due to the bronchial obstruction in anaphylactic shock. (5) Protection afforded by Neoantergan against a shocking dose of antigen in a sensitized animal (Bovet, 1947[1]).

been recorded with Antistine,[29] Hetramine,[37] a derivative of thiodiphenylamine,[44] and α-naphthylmethyl-β-chlorethylamine.[38]

From data given by Loew[65] we have listed the values (TABLE 5) which he obtained in an experiment on passive sensitization, during which the guinea pigs received, successively, a preparatory injection of 0.5 cc. of antiovalbumin serum and a provocative injection of a 0.2 per cent solution of ovalbumin.

In a general way, it is interesting to note that the antihistaminic and antiallergic properties of different products parallel each other closely. This mutual relation is, nevertheless, not absolute. Friedlaender, Feinberg, and Feinberg[66] have compared, in guinea pigs, the antianaphylactic effects of Pyribenzamine and Benadryl. They noted that both drugs, given in identical doses and under the same conditions, afford the same degree of protection, although their antihistaminic properties are very different. The observations made with other species of animals prove that the effectiveness of the various drugs depends upon the nature of the agents producing the shock, a phenomenon similar to that observed with histamine.

TABLE 4

ACTION OF NEOANTERGAN ON THE ANAPHYLACTIC SHOCK PROVOKED IN GUINEA PIGS BY INHALATION OF OVALBUMIN AEROSOL[31]

Dose of 2786 RP mg./kg. s.c.	*Number of animals*	*Average time of exposure to aerosol*	*Results*
0 mg. (controls)	7	3½ minutes	3 sudden deaths 2 violent and reversible shocks 1 pruritus without respiratory difficulties
0.1 mg.	3	10 minutes	respiratory difficulties; not serious
1.0 mg.	6	10 minutes	3 guinea pigs—no symptoms 3 others with dyspnea after 2, 6, and 9 minutes

TABLE 5

ACTION OF 1571 F. AND BENADRYL ON PASSIVE ANAPHYLAXIS ON THE GUINEA PIG

	Dose i.p. mg./kg.	*Number of animals*	*Intensity of shock*						*Mortality*
			0	+	++	+++	++++	*dead*	*%*
Untreated animals	—	45	2	13	6	1	—	23	51
1571 F	3	13	1	1	7	2	—	2	15
Benadryl	3	14	—	5	4	4	1	1	7.1
	12	15	2	6	6	—	—	1	6.6

The antianaphylactic activity of antihistaminic drugs in rabbits has been studied by Pasteur Vallery-Radot, Bovet, Mauric, and Holzer.[69] Diethylaminomethylbenzodioxan and diethylaminoethoxydiphenyl (1262F) protect rabbits regularly against histaminic shock, whereas the action of piperidinomethylbenzodioxan (993F) and thymoxyethyldiethylamine (929F) is less consistent. Campbell and coworkers have noted only negative results with Benadryl.[70] Halpern[26] has made a detailed study of the action of N-ethyl-N-dimethylaminoethylaniline (2325 RP) and of Antergan in the anaphylactic shock in dogs. His results have been confirmed by us[31] with Neoantergan and by Yonkman, Hays, and Rennick[71] with Pyribenzamine.

The anaphylactic syndrome of the dog is characterized by disorders of the blood and blood vessels (hypotension, vasodilatation, and hemoconcentration due to the transudation of plasma through the capillaries), by disorders of the respiration (dyspnea caused by bronchospasm), by the reaction of smooth muscles (intestinal colics, diarrhea, vomiting, and miction), and by nervous disorders (algidity, torpor, hypothermia, and ataxia). The antihistaminic 2325 RP, injected into the dog at a dose of 10 mg./kg. a few minutes after the onset of shock, suppressed the intensity of the manifestations in 60 per cent of the tests, whereas Antergan injected similarly was active in all of the 6 tests.

In some experiments with Neoantergan, the protection was complete even with a dose of 1 mg./kg. (FIGURES 10 and 11). Benadryl affords equal protection against anaphylactic shock in the dog. Wells, Morris, and Dragstedt[72] found no mortality in a group of 22 dogs sensitized with horse serum, while 9 animals died in a group of 26 untreated animals (34.6 per cent). Mayer and Brousseau[73] tried to obtain, with antihistaminics, protection of the mouse against anaphylactic shock provoked by an intravenous reinjection of undiluted horse serum into sensitized animals. They observed a mortality of 89 per cent in the untreated animals and 42 to 70 per cent among the animals previously treated with Pyribenzamine. In no case, however, have mice been protected against the characteristic shock syndrome. Neither Antergan[74] nor Antistine[75] inhibit the formation of antibodies in the animal.

The protection conferred by antihistaminics against anaphylactic shock in the guinea pig extends to a great number of other manifestations of the allergic state. This is particularly true for the so-called "Schultz-Dale reaction." Results of this nature, with 929F, have been published in collaboration with Staub.[10] A portion of the small intestine from a sensitized animal contracts in the presence of the antigen, whereas that of a normal animal does not. These allergic reactions obtained outside the organism present the same specificity and sensitivity as that of the anaphylactic reaction of the whole organism. Introduced at a dilution of 1/2,000,000 into the bath liquid in which a section of the isolated organ of a sensitized guinea pig was immersed, 929F and 1571F inhibited the contraction caused by the antigen in the same way they inhibited the effect of histamine. Rosenthal and Brown[13] confirmed these results while studying the reactions of the guinea-pig uterus sensitized to ovalbumin. Other positive results were obtained by Meier and Bucher.[29]

A similar reaction can be provoked by the addition of allergen to the perfusion liquid of an isolated lung from a sensitized animal. Ellis[76] observed that Benadryl afforded a definite protection, while in other assays, with Pyribenzamine,[77] negative results were obtained, probably due to differences in the intensity of the anaphylactic sensitization.

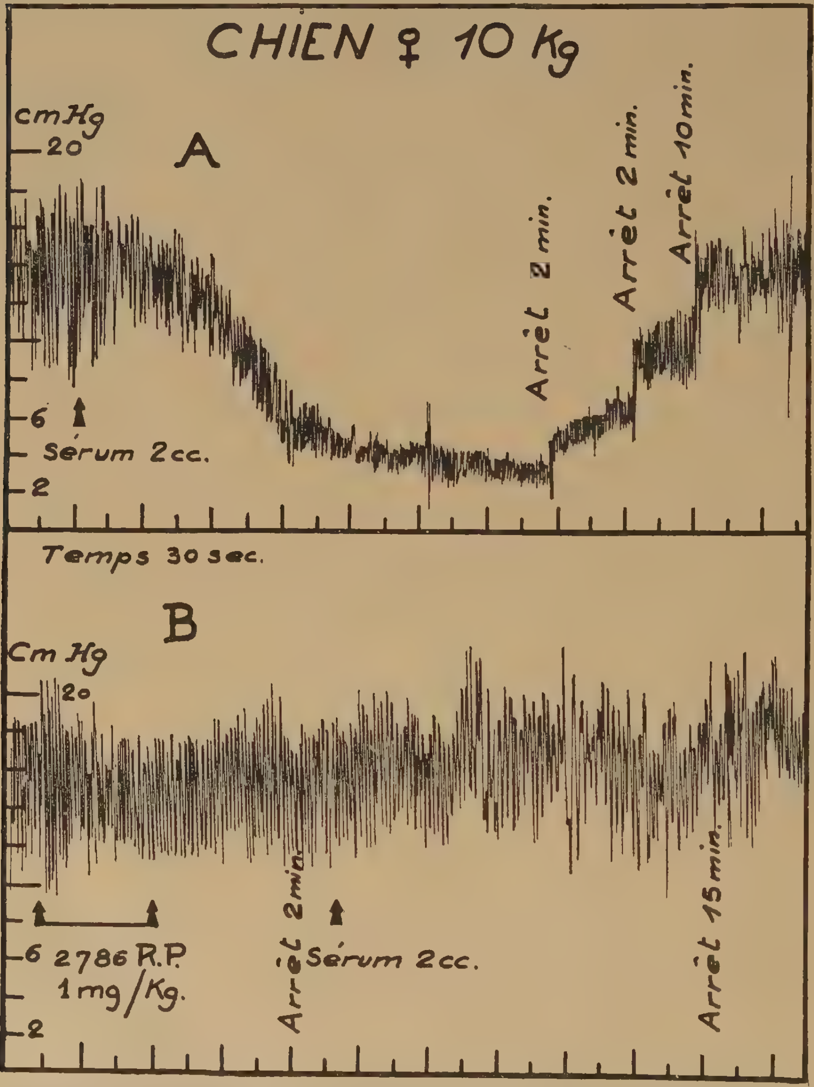


FIGURE 10. Antianaphylactic action of Neoantergan on the dog. Dog: 10 kg. The preventive injection of Neoantergan gave complete protection against shock. Twenty-five days after sensitization by injection of 0.25 cc. i.v. and 0.75 cc. i.m. ox serum, the dog received intravenously a provocative injection of 2 cc. of serum and presented characteristic shock with abdominal spasm, emission of urine, and hypotension. A reinjection of the antigen performed three days later, after a preventive dose of 1 mg./kg. of Neoantergan (2786 RP) had been given, did not produce any anaphylactic reaction (Bovet and Walthert[31]).

Cutaneous allergy represents another field in which synthetic antihistaminics exert a characteristic action. Certain observations made in hu-

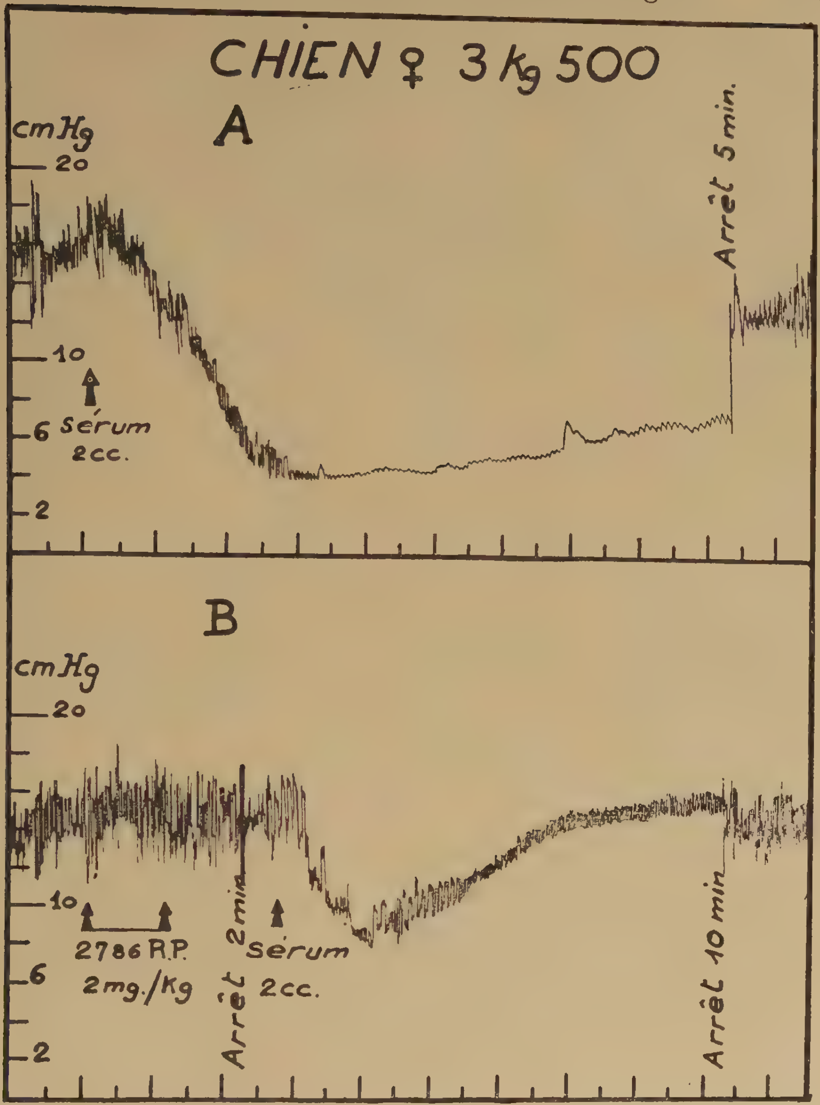


Figure 11. Antianaphylactic action of Neoantergan on the dog (continuation). Dog: 3.5 kg. The preventive injection of Neoantergan considerably attenuates the manifestation of an intense anaphylactic shock. 25 days after sensitization by an injection of horse serum (1 cc. i.v. and 1 cc. i.m.), the dog received intravenously a provocative injection of 2 cc. of the same serum, whereupon it presented severe shock with diarrhea, emission of urine, vomiting, hypotension, and cardiac involvement, followed by a period during which the pulse was hardly perceptible. The number of red cells rose from 5,630,000 to 7,920,000, corresponding to a hemoconcentration of 40 per cent. A second test, performed six days later, clearly showed the protection afforded by Neoantergan when administered a few minutes before the provocative injection of serum. The shock was extremely slight in the protected animal, producing only slight hypotension and no other troubles. The number of the red cells remained unchanged (4,520,000 to 4,760,000) (Bovet and Walthert[31]).

mans have experimental significance, namely, those relative to the reactions produced by the intradermal injection of antigens in sensitive subjects and to the protection produced by the antihistaminics. The first observations of Serafini[78] were confirmed by Friedlaender and Feinberg[79] (Antergan), by Harley[80] (Benadryl), and by Arbesman[81] (Pyribenzamine).

It is possible to reproduce, even in guinea pigs, other typically anaphylactic manifestations which represent cutaneous reactions to the allergen. The activity of antihistaminics against these manifestations is very constant. Mayer[82] points out their regression 15 to 35 minutes after a subcutaneous injection of Pyribenzamine (10 to 25 mg./kg.), while the symptoms reappear when the effect of drug has subsided, namely, 2 to 5 hours after the treatment.

It is apparent from the experiments of Mayer[82] that the evolution of contact dermatitis, which is much less susceptible to the effect of antihistaminics than anaphylactic reactions of the guinea pig, can nevertheless be influenced to some extent. Pyribenzamine attenuates and retards the development of dermatitis caused by para-phenylenediamine or dinitrochlorobenzene in the sensitized guinea pig. Histological examination reveals that the inflammatory reactions, edema, spongiosis, and parakeratosis, do not develop in the treated guinea pig to the same degree as in the untreated animal.[83]

Another aspect of the allergic reaction is the phenomenon of Sanarelli-Shwartzman, namely, the vascular hemorrhagic reaction produced at the level of the dermis. The experiments we shall now mention[31] indicate that the antiallergic activity of the antihistaminic substances, although not so consistent on the cutis as on the smooth muscle contraction, is evident (FIGURE 12).

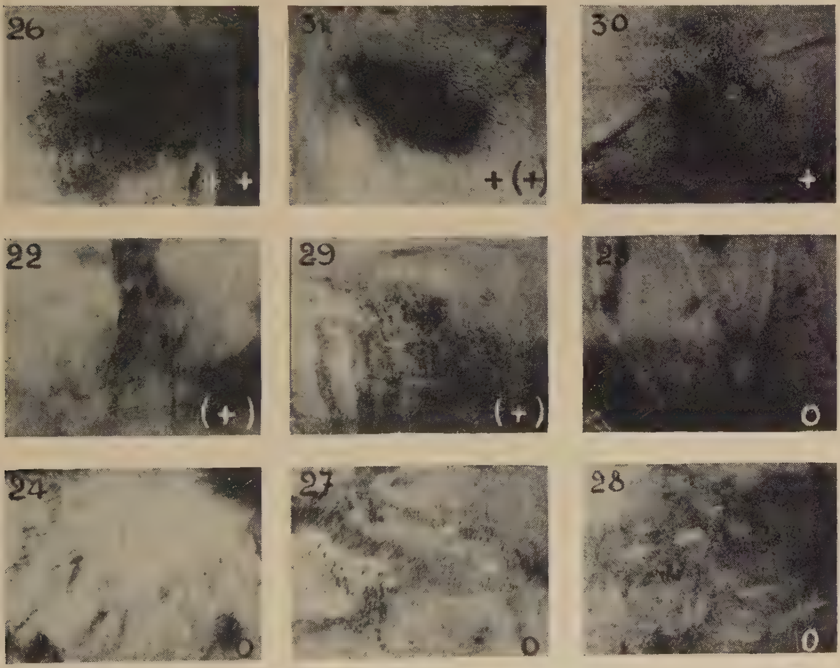
According to the classic technique, rabbits, sensitized locally by intradermal injection, received, 24 hours later, an intravenous injection of allergen. In the areas where the intradermic infiltrations had been produced, some local reactions were now appearing. Clearly visible one hour later, the large ecchymosis and the edemas reached their maximum nearly 6 hours after the injection. The results of the test are given in TABLE 6. All untreated animals presented moderate to strong injury, while 65 per cent of the treated animals showed only slight or no reactions. Boquet[84] has observed negative results in similar tests with thymoxyethyldiethylamine (929F) and Antergan. In our tests, these two products have shown activity, although it was inferior to that of Neoantergan.

It has already been stated that, apart from anaphylactic reactions, histamine is liberated during various toxic injuries. The antagonistic effects of the antihistaminic substances in these cases permit us to elucidate the role of histamine. This is a relatively new field. The first observations in this direction were made in experiments by Sohier (cit.[11]) on the effect of 929F on the general disorders caused by intoxication with dichlorethylsulphide. Halpern[26] has demonstrated the protection against the peptone shock by antihistaminics.

Although the intravenous injection of trypsin in the dog causes symptoms similar to those observed in anaphylactic shock, Benadryl was without effect.[72] Only a slight attenuation of the hypotension was noted. It is not likely that, in this case, the increase in histamine blood level is important enough to account for severity of the reactions observed.

Another example is found in the experiments of Deschiens.[85] A toxin has been isolated from nematodes of the *Ascaris* type, which, according to Rocha e Silva and Deschiens, probably acts by liberating histamine. This observation is corroborated by the fact that Deschiens was able to demonstrate the protective action of Neoantergan against this toxin.

FIGURE 12. Action of Neoantergan on the hemorrhagic reactions of the Sanarelli-Shwartzman phenomenon. The photographs were taken 48 hours after the intravenous provocative injection. Top: 3 control animals; edema and more or less profound ulcerations. Bottom: 9 rabbits subjected to preventive and curative treatment by Neoantergan. The animals received 4 injections of Neoantergan at intervals of 3 hours; the first, 30 minutes before the provocative injection. (Total dose: 3.5 to 7.5 mg./kg.) In two rabbits, the reactions were no stronger than those occurring in the least reactive among the controls; three rabbits presented only insignificant lesions; and the epidermis of the remaining four was unaltered. The signs, 0, (+), +, and ++++ refer to the degree of the skin lesions observed (Bovet and Walthert[31]).



In regard to the effects of drastic purgatives (castor oil, elaterium, globularia, and colocynth), Erspamer and Paolini[86] observed that antihistaminic substances effectively counteract the action of these poisons on the rat. The authors have implicated histamine in the reaction produced by these products. Similar observations concern the antagonism manifested by antihistaminic substances against edema and hyperemia induced by dionine.[87, 88]

Thus, experiments with antihistaminic compounds in animals have revealed widely divergent activities, such as tensional effects, effects on capillary permeability, spasmolytic actions, effects on secretions, and, finally, prevention of anatomo-pathological lesions.

It is not my intention to discuss the therapeutic aspects of antihistaminic compounds. They shall be dealt with thoroughly by others more familiar with this subject. I wish only to emphasize that, from the very beginning, when Antergan was first investigated in France in 1942, the same great diversity of activities was encountered in clinical patients as observed in experimental animals.[89] Clinicians now have at their disposal Benadryl[90] and Pyribenzamine[91] in the United States and Antistine[92] in Switzerland. We shall enumerate only the principal manifestations studied during the clinical experimentation, already representing a wealth of information on the physiopathologic role of histamine.

TABLE 6

ACTION OF NEOANTERGAN ON THE PHENOMENON OF SANARELLI-SHWARTZMAN[31]

	Number of animals	Local reactions				
		none 0	*weak* +	*medium* ++	*strong* +++ *and* ++++	
Untreated animals	10	"	"	"	5	5
Protection by Neoantergan*	20	4	9	4	3	—

* Four subcutaneous injections within 24 hours represent a dose of 7.5 mg./kg. of 2786 R.P. The first injection was made 30 minutes after the intravenous challenge and two other injections three and six hours later, respectively.

Among all allergic affections, the pathogen role of histamine appeared, *a priori*, to be most evident in urticaria, the allergic wheal in acute urticaria presenting a close analogy with the cutaneous histamine wheal. Coinciding with the onset of the urticarial crisis, an increase in histaminemia is generally found. As a matter of fact, the high therapeutic efficacy of antihistaminic substances in the various forms of urticaria constitutes an additional proof of its histaminic nature. In the various forms of allergic urticaria, regardless of their origin, whether digestive or cryptogenic, a satisfactory effect of the treatment and sometimes an almost immediate cessation of the pruritus has been reported.

Antihistaminics act in a remarkable way in the majority of cases of serum disease and dermographism. Besides urticaria, the antihistaminics are active in a multitude of dermatologic affections, particularly in Quincke's edema, in the allergic accidents during chemotherapy, and in various forms of pruritus.

The use of antihistaminics was also suggested in asthma, which, according to Widal, is the model of allergic manifestations, especially since the results obtained in the prevention of experimental histaminic bronchospasm or allergic bronchospasm were quite remarkable. In human therapy, however, in spite of several very spectacular successes, numerous cases of doubtful or uncertain effects have been reported. It can be said that, depending on the product, approximately 15 to 50 per cent of the results are good or very good. At any rate, allowance must be made for the incidence of a large number of nonhistaminic asthma cases due to bronchopulmonary lesions or circulatory disorders, which are secondary phenomena and are brought about without intervention of histamine. Other affections influenced by antihistaminic treatment are hay fever, spasmodic rhinitis, and allergic conjunctivitis.

Before concluding, permit me to add a short remark. At several points in this paper, speaking of one or another chemical series, I have mentioned that certain chemical syntheses were attempted with the purpose of arriving at new sympatholytic, oxytoxic, spasmolytic, or antimalarial agents. Today, the impetus given by the study of the antihistaminic substances far exceeds the original scope of investigations. We now hope to find new physiological properties in those substances which originally were synthesized in our search for antihistaminics. Over many a circuitous route we have noted that certain phenothiazines are not only antihistaminic but also active in Parkinson's disease. With similar intention, we have resumed the study of phenolic ethers, this time seeking a new synthetic curare.

Thus, on each of the squares of that immense checkered table to which I alluded at the beginning of the paper, the pharmacologist throws his die and entertains the hope of its falling on a winning number.

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THE ACTIVITY OF PYRIBENZAMINE AND RELATED COMPOUNDS WITH SPECIAL REFERENCE TO THEIR MODE OF ACTION

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The anti-allergic properties of antihistaminic substances depend to a great extent upon the character, site, and state of the allergic manifestation against which they are employed. We know today with sufficient certitude that the present-day antihistaminics favorably influence hay fever or acute urticaria in 75 to 95 per cent of the treated cases and are much less effective in eczema or contact dermatitis. The reasons for these differences are not well known, but, since the outstanding activity of Pyribenzamine and related substances is their antihistaminic power, the differences in their anti-hay-fever and antidermatitic activities are attributed to the nature of the allergotoxin involved. Everybody supposes that therapeutic efficacy can be expected only in cases where the allergic manifestations result from an intoxication with liberated histamine.

This presentation will discuss whether this reasoning is correct under all circumstances and whether the antihistaminic activity explains the totality of the antiallergic effect. To this end, we shall compare the various pharmacodynamic and antiallergic activities of Pyribenzamine and certain other antihistaminic substances.

This comparison is restricted to the derivatives of ethylenediamine, amino ethanol, and imidazoline, namely, Pyribenzamine, Neoantergan, Benadryl, Antistine, and related compounds. According to our present knowledge, these substances act by the same physiologic mechanism, and the results obtained with one of them should apply to the entire series.

Pharmacodynamics: The Antihistaminic Activity. The principal point of attack of these substances is histamine (that is, in so far as therapeutic importance and degree or specificity of action are concerned).

Although in the preceding papers the pharmacodynamics of histamine has been thoroughly discussed, we shall, nevertheless, briefly summarize some of the most important activities. Histamine has a great number of physiological and pharmacological properties. It excites smooth muscles in many organs and stimulates the activity of various glands and nerve end-organs which perceive pain and other sensations. On the other hand, it paralyzes certain organ systems, including the capillaries and probably the lymphatics, and so forth.

The following histamine effects are counteracted by Pyribenzamine: [1-5] (1) the contraction of smooth muscles of the small intestines, the colon, uterus, the bronchi in guinea pigs and rabbits, and the nictitating membrane of cats; (2) the histamine-induced salivation and lachrymation in cats; (3) the dilatation of capillaries and wheal formation following the local injection, or the iontophoretic application, of histamine in animals and humans; (4) the hypotension which follows the intravenous injection of histamine.

Histamine-induced gastric secretion and hyperchlorhydria are practically unaffected by all known antihistaminics.

In many instances, the antihistaminic activity of Pyribenzamine or related substances is of the same order of magnitude as that of histamine itself. When tested *in vitro* on the isolated guinea-pig gut, one molecule of Pyribenzamine counteracts many moles of histamine. A complete antihistaminic effect can be obtained with doses of .01 micrograms or less per milliliter (of test solution). When introduced into the lungs as an aerosol, a few micrograms of Pyribenzamine will protect for several hours against the lethal effects of histamine aerosol inhalation or intracardially injected histamine. Ten micrograms, injected intra-arterially, stops the histamine-induced salivation in cats. By subcutaneous injection, 3 mg./kg. of Pyribenzamine protect guinea pigs against up to 120 lethal doses of histamine.

The nullification of these histamine effects has therefore been considered as a specific displacement reaction, similar to the action of sulfonamides upon PABA.

Other Pharmacologic Properties. Antihistaminic activity is, however, not the only pharmacologic effect produced by this group of compounds. As with most of the other substances with strong pharmacologic action, their pharmacodynamic activities extend to various other physiologic mechanisms. Pyribenzamine has an atropine-like action on smooth muscles and glands, nullifying the spasmodic effects of acetylcholine. It has local anesthetic activity and, furthermore, excites the central nervous system. Although suspected, it has not yet been shown that certain of these so-called "secondary effects" may be intrinsically linked to "antihistaminic" activity.

These secondary pharmacological activities are, however, relatively minor, and the antihistaminic activity represents, in all substances thus far studied, a high peak in the pharmacological spectrum. The anticholinergic power of Pyribenzamine, for instance, as determined *in vitro* on the guinea-pig gut, amounts to only one one-thousandth of its antihistaminic power. This relationship between antihistaminic and antiacetylcholine activity is not constant, and it varies with the different antihistaminic compounds. All degrees and gradations are encountered between substances with very high antihistaminic and low antiacetylcholine activity, and vice versa. In other words, there exist all degrees of transitions from the antihistaminics of the Pyribenzamine type to the antispasmodics of the Trasentine type. Also relatively weak, as compared to the antihistaminic power, is the local analgesic power of Pyribenzamine and the various related antihistaminics. Their potency is one to three times that of procaine, and in this case too, all gradations of antihistaminics with weak local anesthetic power to strong local anesthetics with small antihistaminic power are known.[6]

Antiallergic Activity. Substances with such high antihistaminic power should exert strong antianaphylactic activity, provided the histamine theory of anaphylaxis is valid. From the pharmacodynamic analysis of the first antihistaminic substances of this group, 929F. and 1571F, Bovet and Staub[7, 8, 9] had already assumed and seen that they prevented the appearance of all manifestations which are produced by the release of histamine in anaphylaxis.

On the other hand, from purely theoretical speculations, one would expect that these compounds would remain ineffective in all allergies in which allergotoxins other than histamine are liberated and are the cause of allergic manifestations. It should similarly be ineffective in those conditions in which histamine is liberated in relatively large amounts but constitutes only a by-product and remains innocuous, since the animals or the organs in which it is liberated are insensitive to histamine.

As far as allergic manifestations are concerned, laboratory experiments and clinical trials have partially confirmed this hypothesis. Antihistaminic substances have proven to be highly active in all those allergic manifestations which most strikingly resemble histamine effects, that is, anaphylaxis of guinea pigs and anaphylactoid symptoms of man, serum disease, acute urticaria, hay fever, and rhinitis. In addition, however, they proved to be active in other allergic manifestations not believed to be produced by histamine. They were indeed quite effective in certain experimental sensitizations of the epidermal type, namely, contact dermatitis, as well as in certain cases of dermatitis and eczema in humans.[10, 11]

It has been said that, with the use of antihistaminics, we could detect what role histamine plays in various pathological processes. If this were true, the activity of Pyribenzamine in epidermal sensitizations could suggest that, contrary to all that is known thus far, histamine is the allergic poison responsible for many cases of dermatitis, eczema, and other inflammations of the skin where histamine release has indeed been demonstrated but, until now, has been considered a waste product. However, the activity of Pyribenzamine in dermatitis may be attributed to another mechanism. It is possible that this substance prevents or alleviates certain allergic manifestations not only because it counteracts histamine, but also through one of its secondary pharmacologic activities.

An investigation of this alternative possibility resolves itself to the questions as to which physiologic mechanism operates in a special case of allergy and whether the so-called secondary pharmacologic properties of Pyribenzamine, such as the local anesthetic, antispasmodic power, or others, still unknown, play a role in certain therapeutic effects wherein histamine is not involved.

Such a study was carried out with various experimental sensitizations in laboratory animals, representing a much more uniform test subject than human allergy. The following allergies were investigated: (1) anaphylaxis of guinea pigs, as an example of primary sensitization of the smooth muscle; (2) anaphylaxis in mice, as an example of allergy in histamine-resistant animals; (3) urticaria in guinea pigs, as an example of sensitization in which the capillary system of the skin is principally involved; and (4) contact dermatitis in guinea pigs, as an example of an allergy in which the epidermal cells are the primary site of the sensitization.

We shall now discuss these experimental sensitizations in detail.

Anaphylaxis of Guinea Pigs. Anaphylaxis of guinea pigs is almost identical with histamine intoxication. Of all forms of experimental allergies, it responds most successfully and promptly to Pyribenzamine in surprisingly small doses. Complete protection from all symptoms of shock is conferred with one subcutaneous injection of 1 to 2 mg./kg. body weight. In fact, one-tenth of this dosage is capable of supressing shock in a number of animals.[2, 12] These borderline doses primarily act on the organs which are most sensitive to histamine, namely, the lung. The guinea pigs die of asphyxia long before any of the other histamine or anaphylactic symptoms have had time to manifest themselves.

Since it seems probable that Pyribenzamine protects guinea pigs from anaphylactic death principally because it nullifies histamine liberated in the lungs during anaphylactic shock, it could be expected that the drug would act in much smaller doses if introduced directly into the lungs as an aerosol. This is indeed the case. The inhalation of a 1 to 2 per cent Pyribenzamine aerosol for ten to thirty minutes protects guinea pigs not only against up to 15 lethal doses of histamine intravenously injected,[13] but also against anaphylactic shock when they are actively or passively sensitized to horse serum. The prophylactic effect may last for several hours. In some instances, an aerosol concentration of only 0.1 per cent Pyribenzamine was effective. The amounts of Pyribenzamine absorbed in the lungs have not as yet been measured, but it can be anticipated that they are of the order of micrograms or even fractions of micrograms (FIGURE 1).

These minute amounts introduced into the lungs selectively protect the smooth muscles of the bronchial system but do not protect other organs. Many animals die of delayed anaphylaxis, or, in the case of histamine injections, show signs of systemic histamine intoxication.

The injection of 10 to 15 lethal doses of histamine into guinea pigs protected with Pyribenzamine aerosol produced a deep narcosis which lasted from 30 to 60 minutes. Similarly, sensitized guinea pigs, protected from anaphylaxis by aerosol treatment, have reacted to the challenge with serum by undergoing a long-lasting state of depression, apparently corresponding to the histamine narcosis. It is entirely possible that certain side effects of antihistaminics, especially the drowsiness and dizziness, are related to this hitherto unknown histamine effect.

Anaphylaxis in Mice. If it is true that the exceedingly high response of guinea pig anaphylaxis to Pyribenzamine is connected with the high sensitivity of this animal to histamine, then it should be expected that Pyribenzamine would display only little or no antianaphylactic effect in the mouse—an animal resistant to histamine.

Mice tolerate up to 750 mg. per kg. body weight of histamine salt, whereas guinea pigs are killed by one one-thousandth of this dose. Mice, unlike guinea pigs, are extremely resistant to any sensitization, and many authors attribute the great difficulty in inducing allergy in them to their low sensitivity to histamine. Since the total histamine content of the normal mouse is approximately 10 mg. of histamine per kg. of body weight and since mice present the first signs of histamine shock only when 100 or more mg. of free base are injected, it is rather improbable that histamine is involved in mouse anaphylaxis.

We were able to show that Pyribenzamine and Benadryl did not counteract histamine intoxication of mice, as is the case with guinea pigs or dogs.[14] On the contrary, they augmented the toxicity of histamine—an observation which recently has been confirmed by Halpern[15, 16] in his toxicity studies with the antihistaminics of the thiodiphenyl series. Anaphylaxis in mice also was essentially unaffected by Pyribenzamine. A slight influence was sometimes observed with near toxic doses, but this effect was not comparable to its high effectiveness in guinea-pig anaphylaxis, where Pyribenzamine was effective in about one four-hundredth of the lethal dose.

This result constitutes additional evidence for the assumption that mouse anaphylaxis is caused principally by an anaphylactotoxin other than histamine. It also confirms the finding that Pyribenzamine counteracts anaphylaxis only when it is induced by histamine. From these experiments, it appears that the activity of antihistaminics in the anaphylactic form of guinea-pig allergy can properly be explained on the basis of a single pharmacologic activity, namely, nullification of histamine effects.

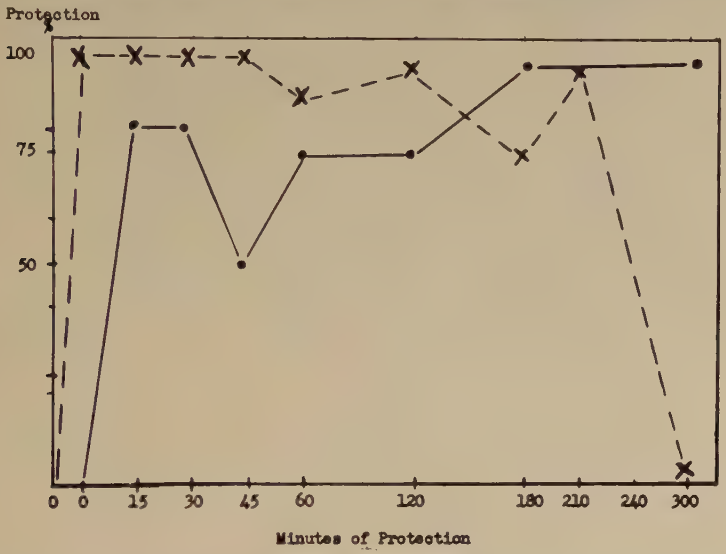

FIGURE 1. Pyribenzamine aerosol. Protection of guinea pigs against histamine intoxication and anaphylactic shock X = histamine; ● = anaphylaxsis). Aerosol: 2 per cent Pyribenzamine by aerosolizatioy as described by Mayer.[2] Inhalation for 30 minutes. Histamine intoxication: 3 mg./kg. histamine phosphate (= 4 lethal doses) intracardially injected at various intervals. Anaphylaxis: Active sensitization to horse serum. Reinjection of 1 ml. of horse serum at various intervals after aerosolization. Abscissa: Time in minutes after the end of aerosol treatment. Ordinate: Percentage of protected inhalation animals. Total number of animals: 173.

Urticaria. Acute urticaria is one of the most responsive clinical indications for antihistaminic therapy. It is assumed, according to all we have learned since the work by Lewis on the urticarial wheal, that the therapeutic effectiveness of antihistaminics in this condition also depends primarily upon the nullification of histamine. Curiously enough, the therapeutic value of the various antihistaminics decreases as the urticaria becomes more chronic, in spite of the fact that the clinical symptoms of acute and chronic urticaria are the same. The question arises whether, in these chronic cases, a shift has taken place from histamine, as the etiologic factor, to another urticariogenic allergotoxin.

The answer to this problem is still unknown. Thus far it has been possible to produce experimentally only acute urticaria, or at least symptoms very close to acute urticaria. When guinea pigs are sensitized to hog serum and challenged 20 days later with an intradermal injection of the same antigen, an urticarial eruption appears within 16 to 24 hours, which is localized particularly on all shaven parts of the skin, on the ears, and on the visible mucosae.[17] The histological picture of the lesions is that of a typical urticarial eruption, with primary changes on the capillary system in the form of dilatation of the blood vessels and lymphatics (FIGURE 2). This rash promptly disappears within 10–15 minutes with Pyribenzamine therapy when doses ranging from 5 to 10 mg. per kg. of body weight are injected, and reappears after an interval of several hours unless treatment with the antihistaminic is repeated.[18] The result of one experiment is given in TABLE 1.

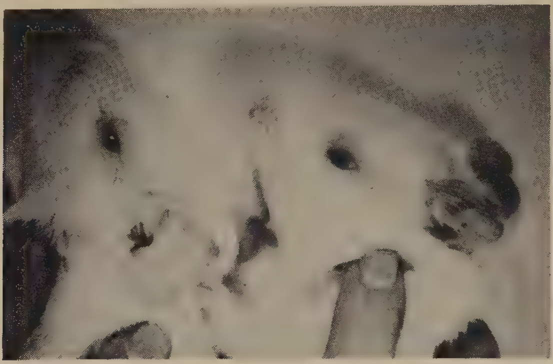

FIGURE 2. Influence of Pyribenzamine upon the urticarial manifestation produced in guinea pigs sensitized to hog serum. The animal on the right is a control animal, challenged with hog serum and presenting deep-red ears and mucosae. The animal on the left was treated in the same manner, but received a subcutaneous injection of 15 mg./kg. Pyribenzamine at the height of the allergic manifestation. The skin eruption disappeared within 15 minutes and the skin became almost normal.

TABLE 1

EFFECT IN GUINEA PIGS OF PYRIBENZAMINE UPON THE VASCULAR SKIN REACTIONS AFTER SENSITIZATION WITH HOG SERUM

	Pyribenzamine doses—mg. per kg.					
	0.1	*1*	*5*	*10*	*20*	*25*
action	none	none	none in one case, active in another	none in one case, active in three others	active in three cases	active in two cases

15 guinea pigs, sensitized to hog serum and treated, after challenge, with various amounts of Pyribenzamine. Minimal active dose is 5 mg./kg. subcutaneous injection.

This acute urticaria of guinea pigs is only one manifestation of a general anaphylactic sensitivity produced by the sensitization with hog serum. If the antigen is injected intracardially and not intradermally into the sensitized animals, they immediately die from anaphylactic shock, and the general anaphylaxis responds to Pyribenzamine as promptly as does the urticaria. This fact, together with the obvious analogy between guinea-pig urticaria and acute human urticaria, makes it quite probable that the therapeutic effect of Pyribenzamine in experimental urticaria results primarily from the nullification of histamine.

Contact Dermatitis. The next form of experimental allergy in which the mechanism of action of Pyribenzamine was investigated is experimental contact dermatitis. We have seen that this form of allergy is quite resistant to antihistaminic therapy, and there are certain facts which may explain this resistance. Allergic dermatitis, in many respects, develops differently from either the anaphylactic or urticarial forms. The allergic manifestations of anaphylaxis or urticaria appear explosively within a few minutes after the introduction of the antigen. The allergotoxin—in this case, histamine—is liberated at once and acts immediately. Its effect is functional; the allergic manifestation is the result of an excitation or a paralysis of physiologic mechanisms.

The epidermal allergic challenge reaction becomes visible only after an incubation of many hours and is characterized by inflammatory changes at the site of the reaction. The slow onset of the epidermal reaction is probably due not so much to a slow liberation of the allergotoxin, but rather to the fact that the reactivity of the epidermal cell is very slow. The epidermal allergic manifestation is not due to a functional change, but to destructive cell injuries.

The very first symptoms of allergic or nonallergic dermatitis, namely, the erythema and pruritus accompanying dermatitis and especially atopic eczema, are probably produced by histamine liberated during the irritation or the antigen-antibody reaction. They are promptly taken care of by Pyribenzamine. The nature of the allergotoxin responsible for the specific cell changes in dermatitis and eczema which follow this initial state is unknown, but it is quite probable that it is not histamine. Histamine intoxication is, as we have seen, typified by a series of very acute symptoms such as wheals, increased secretion of certain glands, pruritus, and spasm of various smooth muscles. These delayed symptoms manifested in dermatitis or eczema are not typical histamine symptoms, and no one has yet succeeded in producing dermatitis with histamine, even after prolonged administration. These cellular changes, which constitute the characteristic changes in dermatitis and eczema—spongiosis, intraepidermal vesicles, cell infiltration into the dermis and cutis, *etc.*, are apparently the consequence of the liberation of other allergotoxins.

Experimental sensitizations of the epidermal type can readily be obtained when guinea pigs are pretreated with various chemical substances such as paraphenylenediamine, dinitrochlorobenzene, poison ivy extract, and many other compounds known to produce contact dermatitis in man. This hypersensitivity of the skin develops under conditions identical with those leading to contact dermatitis in human beings. One or more intradermal injections or application of salve on the eroded skin usually give rise to a generalized hypersensitivity of the entire epidermis.[19, 20]

The manifestations of these experimentally produced types of contact dermatitis in guinea pigs very much resemble those in human beings. After a primary state of erythema and itching, a vesicular dermatitis with infiltration and eosinophilia in the affected tissue develops, which is followed by scaling and full repair.

The influence of Pyribenzamine upon two different kinds of experimental dermatitis has been studied—one produced by sensitization to paraphenylenediamine (FIGURE 3)[19] and the other by sensitization to 2-4, dinitrochlorobenzene.[21] These two allergies show slight differences in their clinical aspect and in the pathological changes. Paraphenylenediamine dermatitis of guinea pigs is characterized by the regular appearance of intraepidermal vesicles and, thus, very closely resembles dermatitis in man. More persistent erythema is characteristic of dinitrochlorobenzene dermatitis and intraepidermal vesicles are very rare.

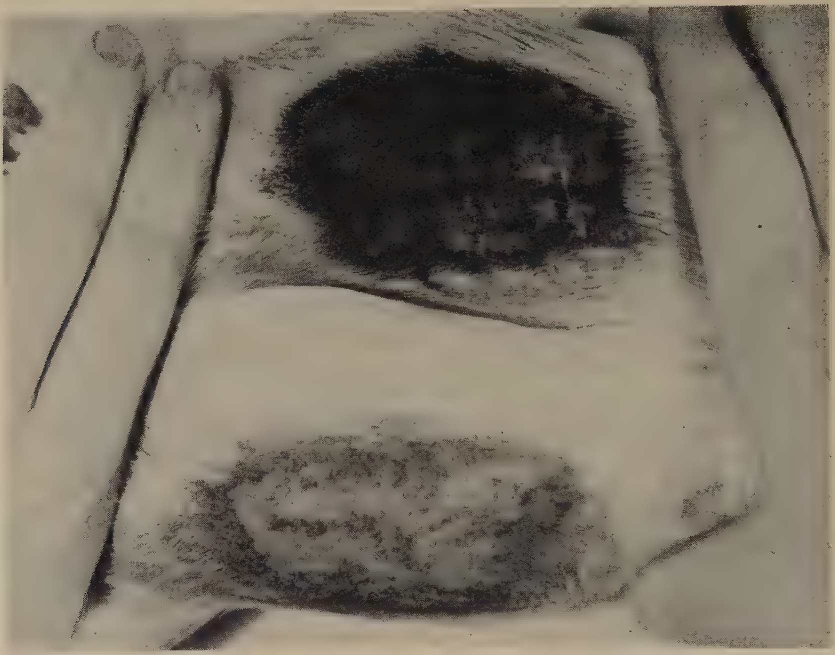

FIGURE 3. Contact dermatitis-type sensitization of guinea pigs to paraphenylenediamine. Top: animal challenged at the height of the sensitization. Thickening and strong infiltration of the skin with eosinophiles, formation of intraepidermal vesicles. In the inflammed skin, large amounts of oxidative enzymes are present, which produce the rapid blackening of the paraphenylenediamine. Bottom: animal treated in the same manner as above animal, but, simultaneously with the challenge, 2 per cent Pyribenzamine base in olive oil was applied locally. The skin is almost normal; infiltration is only slight and shows much less discoloration.

In spite of these differences, Pyribenzamine was therapeutically active in both forms. It reduced considerably the intensity of the inflammation and shortened the healing process. It was effective when doses of 15 to 20 mg.

per kg. of body weight were repeatedly given by subcutaneous injection. It was far more effective, however, when applied locally at the site of the reaction. Solutions of Pyribenzamine base in oil or Pyribenzamine ointment were capable of preventing in a number of animals any signs of dermatitis; in others, they considerably reduced the inflammation. The protocol of one experiment is indicated in TABLE 2. As a consequence of these experiments, the use of Pyribenzamine ointment was proposed for human contact dermatitis and eczema. Lately, Feinberg and Bernstein[22] have reported good results with the topical use of Pyribenzamine.

How can we explain the activity of antihistaminic substances in eczema or contact dermatitis? Shall we suppose, contrary to laboratory evidence, that histamine is instrumental in the production of dermatitis and eczema, as it is in anaphylaxis and urticaria? And shall we believe that high doses of Pyribenzamine and prolonged use are required in dermatitis because considerable cell repair is necessary to restore normality? In an earlier study, we accepted these explanations, but today, as a result of recent studies, we are skeptical. It now seems more probable that Pyribenzamine acts in epidermal sensitizations by virtue of pharmacologic properties different from its antihistaminic power.

TABLE 2

INFLUENCE OF PYRIBENZAMINE UPON EXPERIMENTAL CONTACT DERMATITIS AFTER SENSITIZATION OF GUINEA PIGS WITH P-PHENYLENDIAMINE. PROTECTION AFTER LOCAL TREATMENT WITH 2 PER CENT PYRIBENZAMINE IN OIL

Number of animals	*Pretreatment*	*Pyribenzamine administered*	*Animals showing:*							
			Dermatitis				*Diamine oxidation*			
			++++*	++	+	*0*	++++	++	*0*	(+)
2	None (Not sensitized)	None				2				2
5	Sensitized	None	5				5			
5	Sensitized	2% Pbz. in oil		1	4			1	4	

* + to ++++ represent different degrees of reaction to paraphenylenediamine.

As mentioned in the introduction, the antihistaminic substances are endowed with a great array of pharmacologic properties, in addition to their most striking property of counteracting histamine. Certain of these secondary activities are by no means unimportant attributes for antiallergic agents. There is, for instance, the matter of antiacetylcholine activity. Various theories have been advanced claiming that acetylcholine, rather than histamine, is the ultimate cause of anaphylactic shock or of certain forms of urticaria.

It is not our intention to discuss this theory now, but there are many facts which make it improbable that the antiacetylcholine activity of Pyribenzamine and other antihistaminics plays an important role in any of their therapeutic antiallergic effects. Atropine, Trasentine, and other antispasmodics have no, or at least very little, effect in anaphylaxis and dermatitis. Furthermore, antihistaminic substances with relatively high antiacetylcholine activity, as, for instance, Benadryl, are not stronger antiallergics than substances with low antiacetylcholine activity such as Pyribenzamine. In so far as the role of acetylcholine as a dermatitic agent is concerned, it has not been shown to be more instrumental in producing dermatitis than histamine.

It is more difficult to answer the question as to what extent the local anesthetic properties of antihistaminics may account for their antidermatitic activities. Local anesthetics are known to prevent or diminish anaphylactic shock and dampen other allergic reactions in animals. Similarly, they favorably influence certain forms of allergic eczema, especially the so-called "lichen vidal" forms of atopic eczema. As we have seen, all antihistaminics within the structural family of Pyribenzamine are local anesthetics.

The therapeutic effect of Pyribenzamine aerosols in anaphylaxis is not due to a local anesthetic component, since procaine aerosols were entirely without effect. In dermatitis, the question is not so simple. The local anesthetic activity of Pyribenzamine may well play a certain role when it is applied locally in the form of an ointment, but it is more than questionable whether this activity is implicated when Pyribenzamine is administered parenterally, since no anesthetic or analgesic effect can be observed under these conditions.

It is probable that other, still unknown pharmacodynamic effects of Pyribenzamine are operating in its antidermatitic activity. In investigating this question, two facts may be borne in mind: (1) dermatitis and eczema are general and unspecific inflammatory processes which are not confined to an allergic process; and (2) besides histamine, several other toxic products have been isolated from tissue during inflammation which, unlike histamine, are capable of producing inflammation in normal tissue.[23, 24]

The clinical signs of an allergic dermatitis and eczema are not different from those present in primary, nonallergic irritations of the skin. Since the same pathologic process can result from various and entirely different processes, allergic and nonallergic, it may be assumed that the substances directly responsible for this form of skin inflammation are liberated not only during a primary nonallergic irritation of the skin, but also during an allergic skin irritation following an antigen-antibody reaction.

Various substances have been isolated from primary inflammatory tissue, such as leucotaxine, leucotropine, necrosin, and proteolytic enzymes. A study investigating whether these same substances may also be released and be operative in allergic dermatitis is now in progress. So far, only negative results have been obtained in experiments on the influence of antihistaminic substances upon some of these products connected with inflammation. Cullumbine[25] has investigated the influence of Neoantergan upon leucotaxine, and Loew,[26] that of various antihistaminics upon the skin reaction to trypsin. No effect was observed in either case.

Another enzyme which is released and plays an active role in certain inflammations of skin and other tissues is hyaluronidase. It has been known for a long time that the skin presents a natural barrier to bacterial invasion and subsequent inflammation. The principal component of this barrier is hyaluronic acid, which is present in connective fibers and mucoid tissue. In order to overcome this barrier, invasive bacteria produce or liberate an enzyme, hyaluronidase, which hydrolyzes hyaluronic acid, thus weakening the barrier and permitting invasion of the tissue or spread and diffusion of toxins.[27, 28] Hyaluronidase, however, is not only a product of certain bacteria, but is contained, in bound form, in many cells of the animal organism, from which it is released under certain physiologic conditions. Skin, for instance, is very rich in hyaluronidase.

Various phenomena of spreading are well known in allergic and nonallergic inflammations, especially in dermatitis and eczema. The question therefore naturally arises whether hyaluronidase may be liberated by some nonliving agents and thus play a role in nonbacterial inflammations.

We have studied this problem on guinea pigs sensitized to paraphenylenediamine and challenged later with an intradermal injection of the antigen.

TABLE 3

INFLUENCE OF HYALURONIDASE ON THE CHALLENGE REACTION IN EXPERIMENTAL EPIDERMAL SENSITIZATION TO PARAPHENYLENEDIAMINE (PP) IN GUINEA PIGS

	Number of animals	*Average size and intensity of local reaction*			
		Without hyaluronidase		*With hyaluronidase*	
		Size in cm^2	*Intensity of inflammation*	*Size in cm^2*	*Intensity of inflammation*
Controls..............	4	0.317 ± 0.12	(+)	1.60 ± 0.14	+
Sensitized animals......	18	2.3 ± 0.74	+	16.9 ± 0.52	+++-++++

Challenge: 21 days after sensitzaton, 0.7 mg. of PP in 0.15 ml. of fluid.
Hyaluronidase: 0.7 mg. per 0.15 ml. of liquid injected.
Figures = cm^2 x 10.
(+)–++++ represent different degrees of inflammation (redness + infiltration).

In the presence of hyaluronidase, the size of the allergic, inflammatory reaction was more than 7 times greater than in the absence of this enzyme, which proves that hyaluronidase increases not only the spread of India ink, but also the spread of the antigen, paraphenylenediamine. Of special interest was the further observation that, in spite of the dilution of the antigen in the tissue and the seven-fold increase in reactive area, the inflammatory reaction was not decreased; on the contrary, it was increased several-fold. In the 18 animals tested, the infiltration and the redness were so much more intense that the allergic reaction increased from + to a strength of +++ or ++++ (TABLE 3).

Although it is not possible to draw definite conclusions from these experiments without a study of the comparative hyaluronidase content in normal and inflamed tissue, the results presented are an interesting indication of the possibility that hyaluronidase is involved in this allergic inflammation.

Since this experiment, we have investigated the influence of Pyribenzamine and Antistine upon the action of hyaluronidase[26] and have found that both antihistaminics inhibited the action of the enzyme, upon the spreading of India ink in the rat's skin, by almost 50 per cent—an action similar to that of morphine[30] and salicylic acid.[31] Figures 4 and 5 illustrate the results of an experiment with Pyribenzamine, and Table 4 shows the decrease of the India-ink spread under the influence of Pyribenzamine.

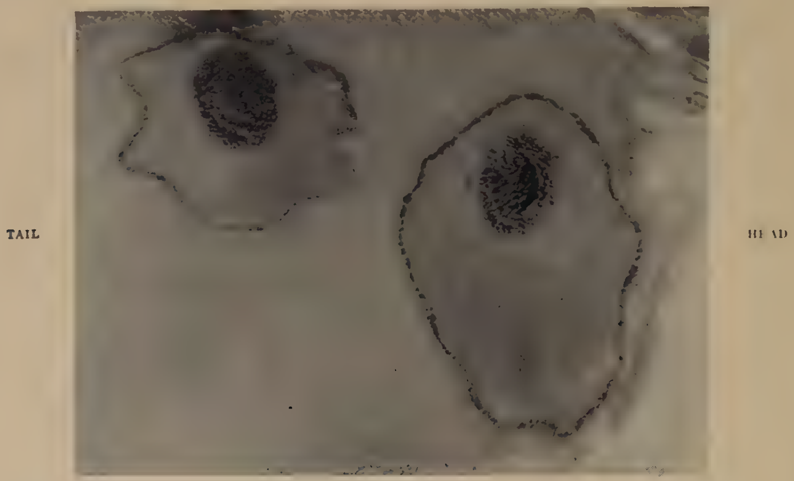

Figure 4. Rat: right side, before Pyribenzamine; tail—India ink plus saline; head—India ink plus 1 per cent hyaluronidase. Dark spot upper center is due to the subsequent injection of Pyribenzamine.)

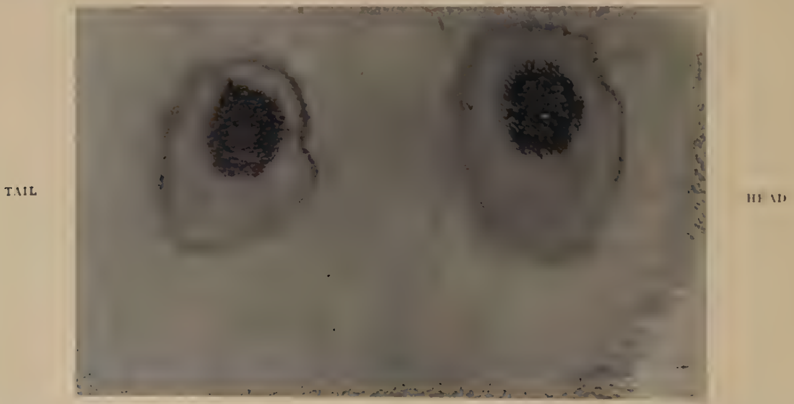

Figure 5. Rat: left side, after 37.5 mg. of Pyribenzamine; tail—India ink plus saline; head—India ink plus 1 per cent hyaluronidase. (This photograph has purposely been reversed in order to permit a comparison of Figures 4 and 5.)

The action of Pyribenzamine upon the hyaluronidase spread of the allergic skin reaction was still more important. This reaction was decreased to almost one-sixth of the controls. In an experiment with 18 sensitized guinea pigs, the reaction size decreased from 17 cm.^{2} and three-plus to almost 3 cm.^{2} and less than one-plus. Table 5 shows the results of this experiment and the comparison with the reaction before Pyribenzamine. This result is interesting and important, since, according to Duran-Reynals,[27] it seems reasonably certain that the spreading effect of hyaluronidase is not connected with histamine. The amounts of Pyribenzamine capable of preventing the effects of hyaluronidase upon the spread of India ink and upon the size and intensity of the allergic skin reactions in animals sensitized to paraphenylenediamine were the same as those which influenced the epidermal contact dermatitis in guinea pigs.

TABLE 4

EFFECT OF PYRIBENZAMINE, ANTISTINE, AND MORPHINE UPON THE SPREADING OF INDIA INK IN THE PRESENCE AND ABSENCE OF HYALURONIDASE

Dosage mg./kg.	*No. of animals tested**	*Per cent reduction of spreading area*	
		Spreading of India ink alone	*spreading of India ink plus hyaluronidase*
Pyribenzamine			
75	20	47.85 ± 2.53	43.95 ± 3.73
37.5	20	18.70 ± 5.50	21.10 ± 3.77
15	20	14.05 ± 4.21	6.25 ± 5.39
Antistine			
75	20	28.10 ± 4.99	37.00 ± 3.22
37.5	20	31.75 ± 5.22	20.00 ± 4.23
15	20	13.65 ± 5.73	11.10 ± 4.77

* Experiments in rats. Each figure represents the mean of the results obtained with 20 animals.

TABLE 5

INFLUENCE OF PYRIBENZAMINE UPON THE ALLERGIC PARAPHENYLENEDIAMINE (PP) REACTION IN PRESENCE AND ABSENCE OF HYALURONIDASE IN GUINEA PIGS

	Number of animals	*Average size and intensity of local reaction*			
		without hyaluronidase		*with hyaluronidase*	
		size in cm²	*intensity of inflammation*	*size in cm²*	*intensity of inflammation*
Controls..............	4	0 (0.317)	0 ((+))	0.75 (1.4)	0 (+)
Sensitized animals......	18	0.27 (2.3)	0 (+)	2.7 (16.9)	± (+++-++++)

Pyribenzamine: 15 min. before and 4½ hours after challenge, 15 mg./kg. subcutaneously.
Figures = πr^2 x 10.
Figures and signs in ( ): results before Pyribenzamine.
The control values represent the mean obtained with 4 animals; those for the sensitized animals represent the mean of 18 animals.

Thus, these experiments have shown that Pyribenzamine and Antistine possess, in addition to their antihistaminic activity, at least one other property which may explain their action in various manifestations not as yet associated with histamine release. The strong anti-hyaluronidase effect, which results in a reduction of the size as well as of the intensity of an allergic inflammation, may well explain their activity in epidermal sensitizations such as eczema or contact dermatitis.

These are the results of the various experiments on the influence of antihistaminics upon experimental sensitizations. Many problems remain unsolved. Nevertheless, we may attempt to answer the question which we raised earlier in our discussion, namely: how might the pharmacologic spectrum of antihistaminics account for their antiallergic activity?

If we recall at this point the various pharmacodynamic properties of the different antiallergic compounds, there is, in our opinion, little doubt that Pyribenzamine acts in anaphylaxis, urticaria, pruritus, and the very first stages of dermatitis by nullification of histamine effects. This action against histamine is the principal pharmacologic and antiallergic property of Pyribenzamine and therefore is the principal basis of its specificity as an antiallergic agent. Other pharmacologic actions, however, are probably responsible for the activity of Pyribenzamine in certain forms of allergic manifestations not connected with histamine.

Dermatitis and eczema are allergies in which, apparently, not histamine, but other allergotoxins are liberated and are responsible for the manifestations. Hyaluronidase appears to be one of these dermatitic allergotoxins. If this be the case, then the antihyaluronidase and anti-inflammatory effect of Pyribenzamine and Antistine may partially or perhaps fully explain their antidermatitic or antieczematic activity.

The mass of literature on the mechanisms of action of antihistaminic substances is growing rapidly. Many facts have been accumulated concerning their pharmacology and antiallergic nature. We are now entering the phase where we find it necessary to deepen and at the same time expand our conception of their mode of action in allergies. As histamine is not the complete answer for allergy, neither does the antihistaminic power entirely explain the antiallergic activity of the so-called "antihistaminic substances." Very few drugs act by a single mechanism. As a rule, the clinical relief which is obtained upon their use is the result of the accumulation of various activities and of simultaneous and concerted attacks upon different cell and enzyme systems. Anithistaminic substances are no exception to this rule, and we believe that they are antiallergic remedies because they attack the allergic process from different directions. There is no doubt in my mind that, in spite of this fact, histamine is the principle point of attack of antihistaminics.

Allergy is one of the most complicated and most varied forms known of all the pathologic processes. It would be too simple to reduce this great complexity to the single formula, histamine. The same consideration applies to antihistaminic substances. At first, the problem was oversimplified and all therapeutic effects were explained on the basis of antihistaminic activity. The more we learn about these substances, however, the more we realize that their antiallergic action appears as complicated and as varied as allergy itself.

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THE PHARMACOLOGY OF BENADRYL AND THE SPECIFICITY OF ANTIHISTAMINE DRUGS

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Designation of compounds as antihistamine drugs, antihistaminics, or histamine antagonists implies that such drugs exert a specific inhibiting influence on the actions of histamine. The term, specificity, as herein used, implies that a drug may exert a definite type of effect, such as antihistamine action, and to a degree which is in sharp contrast to one or more effects which are less conspicuous, not necessarily related and possibly not of pronounced biological significance. The degree of specificity is frequently ascertained (1) by comparing the magnitude of the specific effect of the drug with the lesser effects, all effects being induced by comparable doses of the drug; or (2) by determining the amount of drug necessary for eliciting each effect and noting the relative differences between such amounts, in which case the quantity of drug required to elicit the specific effect should be only a small fraction of that needed to elicit the lesser effects.

Degree of drug specificity is important from several standpoints. A drug which exerts a single type of action can frequently be used effectively as a research tool to produce or prevent a physiological or pharmacological response, the analysis of which the investigator is desirous of undertaking. When specific, proper doses of a drug will evoke the desired response without eliciting other effects to a degree which may render interpretation difficult or impossible. Drugs with a high degree of specificity frequently prove useful as diagnostic agents, since they may enhance or diminish existing dysfunction and their single type of action renders interpretation more reliable. Some valuable therapeutic agents are nonspecific. Nevertheless, from a therapeutic standpoint, a specific drug may prove to be especially useful by eliciting desired effects without adding the complicating feature of untoward reactions which are related to several actions of a drug.[†] The specific effect of antihistamine drugs, as well as their less conspicuous actions, are important from the viewpoint that thorough analysis may provide important information concerning their mode of action.

In substance, the specificity of antihistamine drugs is of importance in relation to their mode of action, their use as research tools, and their application as diagnostic and therapeutic agents. A single example will serve to emphasize the significance of antihistamine specificity. If a reasonable degree of specificity has been demonstrated for antihistamine drugs, their effectiveness in controlling manifestations of experimental anaphylaxis and clinical allergy would constitute definite support for the belief that histamine is an important factor involved in such conditions.

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[*] It is not the intention to imply that specific drugs fail to induce undesired side-effects, since it is common knowledge that a drug as specific as atropine, when used as an antispasmodic, may induce tachycardia, mydriasis, and xerostomia. Such undesired responses are due to the antiacetylcholine action of atropine, a drug which is specific but which lacks selectivity for a given tissue and therefore alters activity in a number of tissues, each of which depends upon the action of acetylcholine for normal activity. By analogy, there is the possibility that a specific antihistamine drug would also induce diverse responses in the animal organism, but this is largely dependent on whether histamine is intimately related to the physiological economy of the animal organism.

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The realization that antihistamine drugs might exert several types of action to some degree stemmed from considerations of chemical structure and pharmacological actions actually demonstrated. The chemical nature of most antihistamine compounds immediately suggests the possibility that atropine-like, antispasmodic, local anesthetic, or sympathomimetic actions might be demonstrable. One of the early reports from Dr. Bovet's laboratory by Staub[55] mentions that the phenolic ethers and ethylenediamine derivatives were systematically examined for sympathomimetic and sympatholytic action. This is an important point, since developments made during the past decade indicate that all antihistamine drugs either enhance or diminish some of the excitatory effects of epinephrine.

The original antihistamine drugs, 929F, 1571F, and Antergan actually exhibited several pharmacological properties other than the antagonism of histamine.[20, 55] Confirmation and extension of the experimental findings with these drugs, as well as evaluation of the antihistamine drugs developed more recently (for refs. *cf.* Loew[33]), reveal the necessity of considering several pharmacological properties in addition to antagonism of histamine. A recent review of the literature[33] reveals that antihistaminics can produce several pharmacological effects. For purposes of orientation and development of the discussion concerning the specificity of antihistamine drugs, the pharmacological properties which appear to deserve consideration in relation to antihistamine action are herein outlined:

(1) An atropine-like action is indicated by the ability of a drug to prevent acetylcholine from exerting its usual secretory, depressor, mydriatic, or spasmogenic action. Only a very low, and probably insignificant, degree of anti-acetylcholine action is revealed in the literature dealing with 929F,[49, 50, 55] 1571F,[20, 38, 55, 58] Antergan,[20] Antistine,[4, 9, 44] Neoantergan,[8, 13, 52, 53] and Pyribenzamine.[41, 53, 61] The same is true of the thiophene analogue of Pyribenzamine studied by several groups of investigators,[17, 29, 32, 51] as well as the halogenated thiophene analogues examined by Litchfield and coworkers.[32] The weak, atopine-like action of Benadryl is less than one-fiftieth or one one-hundredth of that exhibited by atropine itself. Evidence of such atropine-like action of Benadryl includes the antagonism of the spasmogenic effects of acetylcholine[10, 38, 58] and the antagonism of its depressor action.[38, 53] In addition, mydriasis has been demonstrated in rabbits[35] and man,[24, 43] and Benadryl inhibited salivary secretion induced by cholinergic influence in animals[3, 14] and occasionally caused xerostoma in patients. The evidence now available is insufficient to warrant any conclusion concerning the degree of atropine-like action exerted by derivatives of thiodiphenylamine (phenothiazine), described by Halpern,[21–23] or by the compound, Thephorin, developed by Lehmann and associates.[30]

(2) The antispasmodic action of antihistamine drugs appears to be related to the degree of atropine-like action which has just been considered

briefly. It is now quite apparent that general antispasmodic or spasmolytic action is not proportional to the degree of antihistamine action, since potent antihistamine drugs may exert only a weak antispasmodic action or actually induce spasm of smooth muscle.

(3) A spasmogenic action is demonstrable when several ethylenediamine derivatives are added to fluids used to bathe or perfuse smooth muscle of isolated lungs, intestines, or uterus.[13,20,29,41,55,62] Dilutions which induced spasm usually ranged from 1:20,000 to 1:100,000. Higher dilutions failed to induce spasm and were capable of blocking the spasmogenic effects of histamine. In intact animals, spasm of intestines and the uterus has also been observed.[53] The fact that several potent antihistamine drugs have the propensity of exerting a spasmogenic action is an added reason for not classifying antihistamine drugs as general antispasmodics.

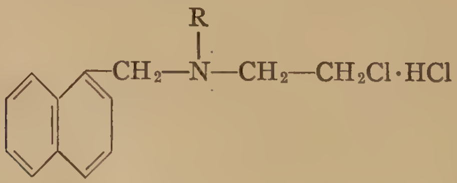
(4) Local anesthetic action is exerted to some degree by all antihistamine drugs, but, unfortunately, very few quantitative studies concerning potency and duration of action have been made. When Benadryl was injected intradermally in man, Leavitt and Code[27,28] found its local anesthetic action to be at least twice as strong as that exerted by Procaine. The local anesthetic activity of Neoantergan was three times that of Procaine as determined by the intradermal test in guinea pigs.[13]

(5) An enhanced and prolonged pressor response to epinephrine occurs (see Loew[33] for refs.) in anesthetized dogs and cats treated with Antergan, Neoantergan, Benadryl, Antistine, Pyribenzamine, and the thiophene analogue of the latter drug.[29] The cause of this phenomenon has not been ascertained, but evidence has been adduced to indicate that the effect is probably unrelated to any atropine-like action.[53] Furthermore, some antihistamine drugs do not elicit this effect.

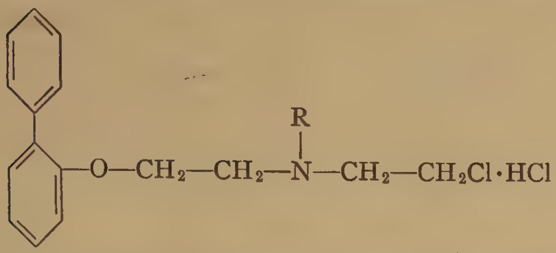
(6) Diminution of the pressor responses to epinephrine occurs in dogs treated with 929F and Thephorin.[7,30] This effect can be considered in conjunction with the fact that compounds have recently been discovered which not only antagonize histamine but also block the pressor action of epinephrine and even reverse the pressor to a depressor response.

(7) Sympatholytic or adrenergic blocking action was found to be a prominent effect of certain N-alkyl derivatives of α-naphthylmethyl-β-chloroethylamine and β-(2-biphenyloxy)ethyl-β-chloroethylamine.[2,33,39] The structural formulae of these compounds are as follows:

R = Alkyl ($CH_3 \cdots C_6H_{13}$)



α-naphthylmethyl-alkyl-β-chloroethylamine·HCl



β-(2-biphenyloxy)ethyl-alkyl-β-chloroethylamine·HCl

These compounds, especially those in the naphthyl series, were also highly effective in diminishing certain effects of histamine. It is possible, however, that they cannot be properly designated as antihistamine drugs, since the adrenergic blocking action was so conspicuous. The ability of these compounds to prevent several effects of histamine and the excitatory effects of epinephrine renders them useful as research tools. For instance, it is inconceivable that the ability of these β-chloroethylamines to antagonize histamine is related to any enhancement of the pressor effects of epinephrine, since these compounds actually reverse the pressor effects of epinephrine.

Admittedly, the drugs which antagonize histamine may elicit one or more of a variety of effects. The degree of specificity against histamine will therefore be dependent on the prominence of antihistamine action in relation to other effects. The most important effects of histamine which have been demonstrated to be diminished or annulled by antihistamine drugs[33] are as follows: (1) spasm of smooth muscle located in the bronchioles, small intestine, and uterus; (2) relaxation of arteriolar smooth muscle, as indicated by a depressor response to histamine or increased vascularity of a region; (3) increased permeability of capillaries, as indicated by wheal formation and transudation of dyes from the vascular system into localized cutaneous areas into which histamine has been introduced; (4) Dilatation of capillaries and other vessels, as evidenced by localized flare reactions and extensive erythematous responses over cutaneous areas.

Antihistamine drugs do not diminish or abolish the effects of histamine on all types of effector cells, since they fail to diminish histamine-induced gastric secretion to a significant degree; and there is not adequate evidence to prove that they diminish the secretogogue action of histamine on pancreatic, lacrimal, and salivary secretory cells.[16, 33]

The diversity of effects of histamine which can be diminished or abolished by antihistamine drugs at once suggests that these agents possess some specific property. It will prove of value to examine detailed evidence concerning the relative specificity of antihistamine drugs in relation to the various effects of histamine.

A. *Smooth Muscle Spasm Induced with Histamine*

(1) *Bronchospasm.* Usually, antihistamine drugs have been selected and partially evaluated by determining their effectiveness in alleviating

severe symptoms or preventing death due to bronchioconstriction, which is so readily induced in guinea pigs with histamine, either administered or liberated during anaphylaxis.

In the experiments designed and executed during the study of Benadryl and related benzhydryl alkamine ethers, various drugs were included in order to assist in determining whether diminution of histamine-induced bronchioconstriction could be accounted for by an antispasmodic, atropine-like, or local anesthetic action. The data in TABLE 1 permit a comparison of the minimal doses of drugs which reduce mortality in guinea pigs subjected to an aerosol of histamine.

It is significant to note that the minimal doses of Neoantergan and Benadryl which protected guinea pigs against histamine are comparatively small (0.025 to 1.5 mg./kg.). Under these experimental conditions, Pyribenzamine[53] and its thiophene analogue[29] were more effective than Benadryl, whereas Antergan exhibited about equal effectiveness.[34] In short, all the recently developed antihistamine drugs were effective in doses less than 1.5 mg./kg.

TABLE 1

MINIMAL DOSES OF VARIOUS DRUGS WHICH REDUCE SEVERITY OF HISTAMINE SHOCK IN GUINEA PIGS

Drug	*Route*[*]	*M.E.D.* (*mg./kg.*)	*Remarks*
Benadryl·HCl	i.p.[†]	1.5	effective at low dose
	s.c.	0.5	
Neoantergan·H_3PO_4	i.p.[†]	0.075	effective at low dose
	s.c.	0.025	
Atropine·SO_4	i.p.[†]	15.0	low effectiveness
Trasentin·HCl	i.p.[†]	>50.0	noneffective
Procaine·HCl	i.p.[†]	>25.0	noneffective
Epinephrine·HCl	i.p.[†]	0.1	partial protection with toxic dose
α-Naphthylmethylethyl-β-chloroethylamine·HCl s.c.		0.025	pronounced antagonism of histamine with a potent adrenergic blocking drug

[*] Intraperitoneal and subcutaneous doses administered 15 and 30 minutes, respectively, before exposure to histamine aerosol.

[†] Data from J. Pharmacol. & Exper. Therap. **86**: 1,1946 and **89**: 247, 1947.

This protective action cannot be due to an antiacetylcholine action of the antihistamine drugs. First, a comparatively large dose of atropine (15.0 mg./kg.), an exceedingly potent antiacetylcholine agent, conferred only a low protective action in the animals (TABLE 1). This might be ascribed to the antispasmodic action and the direct antagonism of histamine, which has been demonstrated frequently with large amounts of atropine. Secondly, when tested on isolated intestinal muscle, the antispasmodic, Trasentine, exerted more antiacetylcholine action than Benadryl,[38, 58] yet a large dose of Trasentine failed to prevent or relieve histamine-induced bronchioconstriction.[36] Thirdly, Castillo and de Beer[10] have demonstrated that Benadryl in a dilution of 1:15,000,000 exerted anihistamine action when tested on smooth muscle of tracheal rings, which allegedly reacted in a manner similar to smooth muscle of bronchi. However, sixty times as much Benadryl was required to prevent the spasm induced with acetylcholine, and even larger amounts of Benadryl failed to exhibit an antispasmodic action by relaxing the undrugged tracheal muscle. These facts permit the conclusion that the ability of Benadryl to reduce histamine-induced spasm of bronchioles and bronchi is not due to an antiacetylcholine action. Furthermore, Benadryl probably exerts more antiacetylcholine action than several other antihistamine drugs,[13, 20, 41] so it is obvious that the arguments just stated strongly support the belief that a specific antihistamine action explains the protective action of 1571F, Antergan, Neoantergan, Benadryl, and Pyribenzamine. Halpern[20] also demonstrated that Antergan was highly effective in relieving bronchioconstriction due to histamine, whereas it was of low effectiveness in reducing bronchioconstriction induced with acetylcholine in eserinized guinea pigs.

The local anesthetic action of antihistamine drugs, even if especially prominent, could scarcely account for the diminution of histamine-induced bronchioconstriction, since cocaine, Nupercaine, and Procaine (TABLE 1) all failed to prevent the bronchioconstriction in intact guinea pigs.[36] The antispasmodic, Trasentine, also exerts a strong local anesthetic action,[19] but it failed to prevent bronchioconstriction.

Sympathomimetic drugs such as ephedrine and epinephrine cannot be classed as antihistamine drugs, since their ability to diminish various effects of histamine is due to their strong bronchiodilator and vasoconstrictor actions and their ability to decrease capillary permeability. These actions directly oppose those of histamine, which constitutes a physiological antagonism which has not been demonstrated with antihistamine drugs.

Although epinephrine is an exceedingly potent bronchiodilator agent, it failed to relieve histamine-induced bronchioconstriction except with doses so large as to induce symptoms of epinephrine intoxication. No dose of epinephrine was found which would prevent death in all guinea pigs treated with histamine, whereas well-tolerated doses of several antihistamine drugs conferred full protection. Halpern[22, 23] has recently pointed out that antihistamine drugs were more effective than epinephrine and Aleudrine in preventing histamine intoxication in guinea pigs. The evidence indicates that antihistamine drugs specifically antagonize histamine, whereas the conspicuous but nonspecific bronchodilator and bronchiodilator action of epinephrine accounts for antagonism of barium, acetylcholine, and histamine. The antispasmodic or bronchiodilator action of Benadryl must be negligible, since it was just perceptible when large doses (10 mg.) were infused through isolated guinea-pig lungs,[15] no antispasmodic action was demonstrated on tracheal rings,[10] and Benadryl failed to increase the vital capacity in asthmatic patients.[31]

There is no convincing evidence that certain antihistamine drugs exert a sympathomimetic action. Ethylenediamine derivatives and Benadryl increase the magnitude and duration of the pressor response to epinephrine in anesthetized dogs (see Loew[33] for refs.). The cause of this response remains unexplained. Even if it is proven that the phenomenon is an expression of a weak sympathomimetic action, it would not account for the strong antihistamine action of Benadryl and ethylenediamine derivatives. We have demonstrated[2] that α-naphthylmethylethyl-β-chloroethylamine was exceedingly effective in alleviating both histamine shock (TABLE 1) and anaphylactic shock, even though this compound not only failed to exert sympathomimetic action but suitable doses in dogs actually blocked the excitatory effects which follow injections of epinephrine and stimulation of adrenergic nerves.

Lehmann *et al.*[30] have reported that Thephorin decreased the severity of histamine-induced bronchioconstriction. No significant degree of sympathomimetic action has yet been demonstrated with Thephorin, whereas it did diminish the pressor response to epinephrine in cats, a fact which the author has confirmed in dogs.[34] The pressor effects of epinephrine in dogs was also diminished by 929F,[7, 11] but the effect may have been nonspecific and related to hypotension.

Thus, an antagonism of histamine has been demonstrated with 929F, α-naphthylmethylethyl-β-chloroethylamine, and Thephorin. Thephorin and 929F diminished the pressor action of epinephrine, and the naphthyl derivative even reversed such pressor action and blocked or reversed effects of adrenergic nerve stimulation. On the other hand, a number of antihistamine drugs enhance the pressor responses to epinephrine. Thus, antihistamine action is probably independent of either a sympathomimetic or adrenergic blocking action.

In summary, it becomes apparent from the pharmacological data relating to responses of bronchiolar smooth muscle that various drugs exhibit a demonstrable degree of antihistamine specificity, since the antagonism of histamine cannot be accounted for by atropine-like, antispasmodic, local anesthetic, sympathomimetic, or adrenergic blocking (sympatholytic) action. Convincing support for the thesis that antihistamine drugs are relatively specific is the fact that no pharmacological action other than histamine antagonism has been demonstrated in intact guinea pigs with the small doses of drugs required to prevent the bronchioconstrictive action of histamine.

(2) *Intestinal Spasm.* Additional information concerning the relative specificity of antihistamine drugs has been secured by determining their ability to diminish intestinal spasm induced with histamine, acetylcholine, and barium. The early studies with 929F and 1571F revealed that these drugs diminished the spasmogenic effects of histamine, and, from reports by Staub,[55] Halpern,[20] and Climenko *et al.*,[11] it is apparent that they are not absolutely specific, since suitable quantities of the drugs also antagonize the spasmogenic action of acetylcholine and barium. The degree of specificity can be expressed as the ratio of the amount of drug required to antagonize acetylcholine or barium to that required to antagonize histamine.

Thus, Halpern[20] demonstrated that it required more than one hundred times as much Antergan to antagonize barium and acetylcholine as was needed to antagonize histamine. Such relationships can be derived from the data obtained by the author and co-workers[38, 58] when comparisons were made of the effects of Benadryl, 1571F, and several antispasmodics on isolated guinea-pig ileum. FIGURE 1 illustrates the relative degree of effectiveness of Benadryl, 1571F, and atropine in diminishing contractions induced by adding histamine, acetylcholine, or barium to the isolated guinea-pig ileum. Benadryl exhibited an appreciable degree of antihistamine specificity, as indicated by the fact that it required 12.5 and 400 times as much Benadryl to antagonize acetylcholine and barium, respectively, as was needed to antagonize histamine. Results with 1571F indicated greater antihistamine specificity, since it required 33 times as much 1571F to antagonize acetylcholine and 400 times as much 1571F to antagonize barium as was needed to antagonize histamine. Thus, Benadryl exhibited greater antihistamine potency but less antihistamine specificity than 1571F.

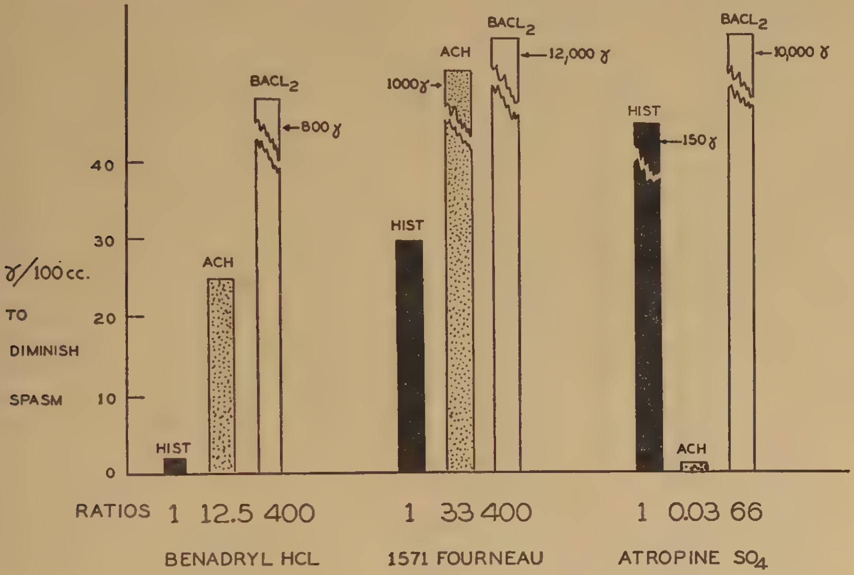


FIGURE 1.

Atropine has long been recognized as a potent and highly specific anti-acetylcholine drug. Minute quantities antagonize acetylcholine but the specificity is not absolute, as indicated by the fact that atropine antagonizes histamine and barium when the amounts of atropine are increased 300 and 20,000 times, respectively (FIGURE 1).

It can be concluded from the effects of 1571F and Benadryl on isolated intestinal muscle that a moderate degree of antihistamine specificity has been demonstrated. A similar degree of antihistamine specificity has been demonstrated for Benadryl by Castillo and de Beer,[10] who made extensive comparisons of drug antagonism on tracheal muscle of the guinea pig. With intestinal muscle, the degree of antiacetylcholine action exerted by Benadryl was much weaker than that exerted by gastrointestinal antispasmodics such as Pavatrine and Trasentine.[38] However, it probably would exceed that exerted by Neoantergan and Pyribenzamine. The information available indicates that Pyribenzamine and Neoantergan only weakly antagonize the spasmogenic action of acetylcholine, and, in view of the strong antihistamine action demonstrated,[8, 41, 42, 53, 59] it is highly probable that quantitative studies would readily provide convincing evidence of a relatively high degree of antihistamine specificity. A high degree of potency and specificity has been demonstrated with Neoantergan.[52]

Attention is drawn to a paper by Schild,[52] which describes methods for determining and expressing both the potency and specificity of antagonistic drugs. Experiments with guinea-pig ileum revealed that Benadryl was 50 times as active against histamine as it was against acetylcholine. Of particular interest is the demonstration that Neoantergan was more potent and more specific against histamine that atropine was against acetylcholine.

The evidence available to date does not indicate that any of the antihistamine drugs has a pronounced ability to diminish the spontaneous motility and tonus of smooth muscle either *in vitro* or *in vivo* (cf. Loew[33]). The drugs, in moderate quantities, usually fail to relax the undrugged smooth muscle of the bronchioles, intestine, or uterus. Thus, the ability to antagonize histamine cannot be ascribed to a general antispasmodic action. Staub[55] and Halpern[20] emphasized the fact that, with 929F, 1571F, Antergan, and related compounds, there was no close correlation between the antihistamine action and the antispasmodic effects. Benadryl does exert a weak antispasmodic effect on intestinal muscle.[38, 58] However, Neoantergan and Pyribenzamine are practically devoid of antispasmodic action. In fact, doses of moderate size may even induce a spasm of uterine or intestinal muscle, *in vitro* and *in vivo*.[13, 20, 29, 41, 55, 62]

(3) *Uterine Spasm.* Nearly all the antihistamine drugs have been demonstrated to antagonize the spasmogenic action of histamine on the uterus in several animal species either *in vitro* or *in vivo*.[13, 20, 29, 41, 53, 55] No data are available, however, which concern the relative degree of action of these drugs in antagonizing other spasmogenic agents such as acetylcholine, barium, and pituitrin. Thus, the degree of antihistamine specificity related to uterine responses remains undetermined.

There has been no demonstration that antihistamine drugs decrease the spontaneous motility and tonus of the uterus, which means that no antispasmodic action has been demonstrated. On the other hand, contraction of uterine muscle has been induced by nearly all of the ethylenediamine derivatives, either when low dilutions were used on isolated uterine tissue[13, 20, 29, 41, 55] or when doses of 3.0 mg./kg. were injected intravenously in dogs.[53]

It may be concluded from the limited amount of data available, and the absence of contrary evidence, that proper doses of antihistamine drugs prevent the spasmogenic action of histamine on the uterus without inducing a concomitant antispasmodic action. Thus, the antihistamine action is not dependent on a general antispasmodic action, especially since most ethylenediamine derivatives, in moderate or large doses, actually exert spasmogenic action.

Further studies with uterine muscle are needed to determine whether antihistamine drugs are relatively specific in antagonizing the oxytocic action of histamine. To the author's knowledge, none of the antihistamine drugs developed in recent years have been tested to determine whether they are capable of antagonizing the spasmogenic effects of pituitrin on uterine and other types of smooth muscle. Studies with pituitrin would extend the knowledge concerning antihistamine specificity and would either support or detract from the degree of specificity established thus far. The natural occurrence of pituitrin in the body is a cogent reason for determining whether widely used antihistamine drugs are capable of altering the role which pituitrin may play in the bodily economy.

B. *Vascular Effects of Histamine As Measured by Changes in Blood Pressure*

The precipitous, transient, decreased blood pressure induced with small intravenous doses of histamine and acetylcholine is largely due to arteriolar dilatation accompanied by some capillary dilatation. It is pertinent to note that the depressor effect of small doses of histamine is largely due to a *relaxing* effect upon vascular smooth muscle and that this relaxing effect of histamine is diminished by antihistamine drugs. It has been demonstrated that the depressor responses elicited by small doses of histamine were diminished but seldom annulled by Antergan,[20, 40, 47] Neoantergan,[5, 6, 8, 47, 53] Benadryl,[38, 40, 53, 57] Pyribenzamine[40, 41, 53, 60] and its thiophene analogue,[29] and Thephorin.[30] Comparative studies made by Marsh and Davis[40] and by Sherrod, Loew, and Schloemer[53] revealed that Benadryl was no less effective than Antergan, Neoantergan, and Pyribenzamine in antagonizing the depressor action of histamine in dogs. Furthermore, a comparison of the doses of drugs used by various workers to diminish the depressor effect of histamine in dogs or cats does not reveal evidence of any pronounced difference in potency among the antihistamine drugs considered.

Pertinent to the present discussion is the fact that certain antihistamine drugs also diminish the depressor effect of acetylcholine. Furthermore, several types of antihistamine drugs enhance the pressor effects of epinephrine, whereas other types either diminish or actually block and reverse the pressor effects of epinephrine. Thus, it is at once apparent, from studies based upon vascular reactions, that antihistamine drugs are not absolutely specific. It is important, therefore, to consider the relative degree of antihistamine specificity and also the information relating to one or more other drug effects which might be somewhat related to the antagonism of the vascular actions of histamine.

In general, the dose of each antihistamine drug (1.0 to 5.0 mg./kg., *i.v.*) most frequently used to demonstrate diminished depressor responses to histamine in anesthetized animals has usually been sufficient to affect the responses to either acetylcholine or epinephrine. This fact suggests a relatively low degree of antihistamine specificity as measured by blood pressure changes, although, admittedly, there have been very few studies[38] in which gradation of doses has been employed to determine the minimum amount of antihistamine drug required to alter responses to substances other than histamine.

Sufficient data are available to demonstrate that diminution of the depressor effect of histamine after treatment with antihistamine drugs is not always coincident with, and therefore not dependent upon, one or more other phenomena. Some of the pertinent facts are indicated in TABLE 2.

Antergan and Pyribenzamine both diminished depressor responses to histamine, but this effect can scarcely be dependent upon an atropine-like action, since the depressor effects of acetylcholine were not diminished. Results with Pyribenzamine[40, 41, 53, 60] are typical of those obtained with its thiophene analogue[29] and with Neoantergan.[5, 6, 8, 53]

TABLE 2

EFFECTS OF ANTIHISTAMINE DRUGS ON VASCULAR RESPONSES IN ANESTHETIZED DOGS AND CATS

Compound	*Depressor effect of histamine diminished*	*Depressor effect of acetylcholine diminished*	*Pressor effect of epinephrine*		
			enhanced	*diminished*	*blocked or reversed*
Antergan·HCl	+	−	+	−	−
Pyribenzamine·HCl	+	−	+	−	−
Benadryl·HCl	+	+*	+	−	−
929F	+	+*	−	+	−
Theporin tartrate	+	+*	−	+	−
α-Naphthylmethylethyl-β-chloroethylamine·HCl	+	+*	−	+	+

* Only a weak atropine-like action has been demonstrated.

Doses of Benadryl (1.0 and 3.0 mg./kg., *i.v.*), used to demonstrate the diminution of depressor responses to histamine, were also effective in reducing but not abolishing the depressor response to acetylcholine.[38] The anti-acetylcholine action of Benadryl, as demonstrated by blood pressure responses, was relatively weak, since it required 1/100 as much atropine to duplicate the effect.[38] Other chemical types of antihistamine drugs, as represented by 929F, Thephorin, and α-naphthylmethylethyl-β-chloroethylamine, do not exert any pronounced atropine-like effects on the vascular system.

Thus, only a low degree of atropine-like action has been demonstrated with some antihistamine drugs, and the ethylenediamine compounds such as Antergan, Neoantergan, and Pyribenzamine were practically devoid of such action. Even when present, the atropine-like action is weak and certainly does not account for the antagonism of histamine, since one has only to recall that the action of histamine is frequently demonstrated and quantitated by using atropinized test objects of high sensitivity, *i.e.*, by measuring contractions of the atropinized, isolated intestine or by measuring depressor responses in the atropinized cat.

In summary, these comparisons of vascular responses indicate that ethylenediamine derivatives of aniline (Antergan) and α-aminopyridine (Neoantergan, Pyribenzamine, and its thiophene analogue) exert antihistamine action without antagonizing the depressor effects of acetylcholine. Benadryl exhibited less specific effects related to blood pressure responses, since the depressor responses to both histamine and acetylcholine were diminished, although the atropine-like action of Benadryl was no more than 1/100 that of atropine itself. The antiacetylcholine action of α-naphthylmethylethyl-β-chloroethylamine and Thephorin was weak, or not demonstrated, with doses employed to antagonize histamine, although more data relating to these compounds are needed before drawing conclusions concerning degree of atropine-like action exerted. It is very unlikely that the ability to antagonize the depressor effect of histamine is dependent to a significant degree upon an atropine-like action. When such action is readily demonstrable, however, it detracts from the degree of antihistamine specificity.

The ability of antihistamine drugs to alter the pressor effects of epinephrine must also receive consideration with respect to the problem of specificity and in relation to the mode of histamine antagonism. As indicated in TABLE 2, it has been demonstrated that Antergan,[12, 46] Neoantergan,[53] Pyribenzamine,[53, 60] and Benadryl[38, 53] enhance the pressor response to small doses of epinephrine in anesthetized animals. Comparable results have been obtained with the thiophene analogue of Pyribenzamine[29] and quaternary derivatives of Benadryl.[54] Although the enhanced pressor response to epinephrine has frequently occurred concomitantly with the diminished depressor response to histamine, there are definite reasons for believing that histamine antagonism is not dependent upon the enhanced pressor response to epinephrine.

First, small doses of Benadryl antagonized histamine without altering the response to epinephrine.[38] In cats, Yonkman *et al.*[61] injected small doses of Pyribenzamine, which sufficed to antagonize histamine but which frequently failed to alter responses to epinephrine.

Secondly, several compounds which diminish the depressor effects of histamine not only fail to enhance the pressor effect of epinephrine but actually diminish the pressor effects (TABLE 2). Thus, 929F and Thephorin antagonize histamine, Thephorin being quite active, and both drugs diminish the pressor response to epinephrine.[7, 11, 30]

Even more striking is the demonstration[2] that α-naphthylmethylethyl-β-chloroethylamine strongly antagonized the depressor effect of histamine in dogs and completely reversed the pressor response of epinephrine to a depressor response. The ability of this naphthyl compound to diminish the depressor response to histamine and to reverse the pressor effect of epinephrine is illustrated by FIGURE 2.

In summary, data relating to alterations in blood pressure reveal that all drugs considered which diminished the depressor response to histamine were capable of altering the blood pressure to epinephrine. However, some of the drugs which antagonize histamine enhanced the pressor effect of epinephrine, whereas others either diminished or actually reversed the pressor response to epinephrine. It is therefore difficult to conceive that either enhancement or reversal of the pressor response to epinephrine could account for the antagonism of histamine. Furthermore, small doses of Benadryl and Pyribenzamine antagonized histamine without significantly altering the pressor response to epinephrine. This is further evidence that the histamine antagonism was not necessarily dependent upon a mechanism which involved enhancement of epinephrine effect. The failure of small doses of Benadryl and Pyribenzamine to alter responses to epinephrine also reveals some specificity of antihistamine action, especially with Pyribenzamine, since it also failed to exert an antiacetylcholine action.

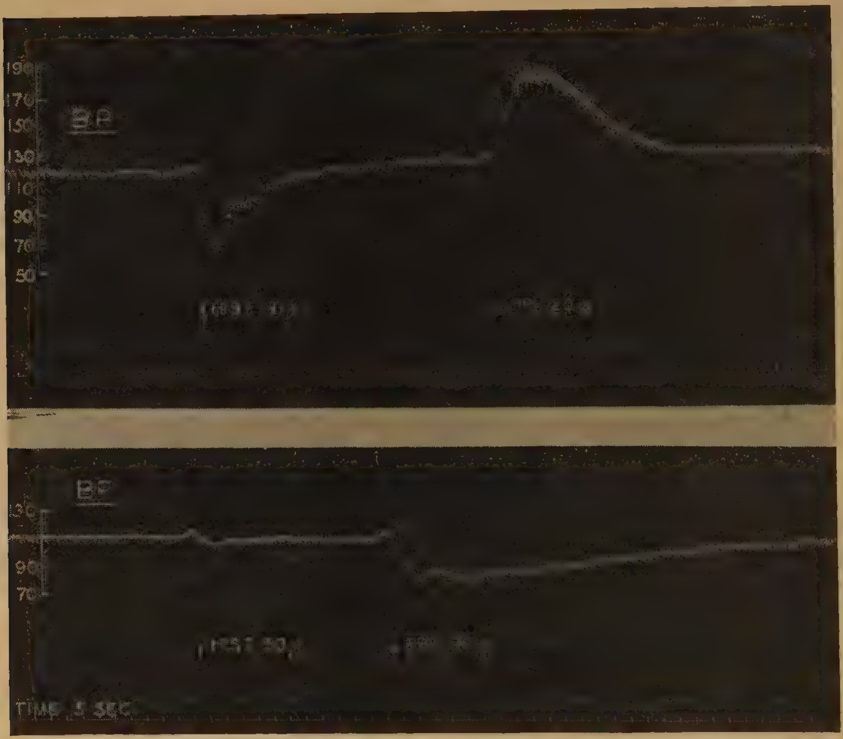


Figure 2. The effect of α-naphthylmethylethyl-β-chloroethylamine hydrochloride on vascular responses to histamine and epinephrine. Record of carotid blood pressure of dog under sodium pentobarbital anesthesia. Above: Control responses. Depressor effect of histamine diphosphate (50γ) and pressor effect of epinephrine hydrochloride (20γ). Below: Record taken 10 to 20 minutes after intravenous injecton of the drug in a dose of 3.0 mg./kg. Note blocking of the depressor action of histamine and reversal of the pressor action of epinephrine.

C. *Localized Cutaneous Responses to Histamine*

The experimental studies concerning histamine-antihistamine antagonism related to capillary dilatation and increased capillary permeability are almost wholly concerned with observations and measurement of the cutaneous responses to histamine. The effectiveness or noneffectiveness of drugs in diminishing localized cutaneous reactions to a variety of stimuli provides information concerning their antihistamine specificity. Following intradermal injection, various substances induce a localized edema or wheal response, which is due, for the most part, to increased capillary permeability which is quite independent of nervous factors. The accompanying flare, on the other hand, is largely dependent upon vasodilatation effected through axon reflex action. Analysis of the wheal and flare response, as influenced by antihistamine drugs, must include consideration of influences on increased capillary permeability, vasoconstriction, vasodilatation, and the nervous factors related to the flare reaction.

It is not likely that changes in vascularity *per se* induced by antihistamine drugs would account for diminution of the wheal and flare response to histamine or other substances, since neither vasodilator nor vasoconstrictor action of definite degree has been demonstrated with antihistamine drugs. Furthermore, as will be more apparent in the later discussion, if a vasodilator or vasoconstrictor action accounted for diminution of histamine effects, then cutaneous responses to numerous other agents should be diminished, which is not the case.

Apparently, all antihistamine drugs exert some degree of local anesthetic action.[9, 11, 13, 20, 27, 28, 37, 41, 50, 55] Injection in localized areas or topical administration of suitable quantities could affect cutaneous reactions by virtue of local anesthetic action. Friedlaender and Feinberg[18] have completely ignored the probability that local anesthetic action of Benadryl (5 per cent) applied topically to scratched surfaces of human skin accounted, in part, for the reduced flare and wheal responses to histamine, codeine, and allergens. Local anesthesia is known to block the axon reflex and thus diminish the flare reaction. Diminished vascularity, in turn, might indirectly diminish the wheal response. Experiments made by Leavitt and Code[27, 28] demonstrated several important facts related to the properties of Benadryl and the interpretation of its effect on cutaneous reactions. Intradermal injections of Benadryl in man induced an appreciable degree of cutaneous anesthesia. In addition, cutaneous responses to histamine were definitely diminished by both Benadryl and Procaine.[28] After the local anesthetic effect of Benadryl and Procaine had disappeared, however, some other action of Benadryl persisted and diminished the cutaneous reaction to histamine. Thus, the local anesthetic action of Benadryl did not account for the persistent inhibitory influence on the cutaneous reaction to histamine and it is probable that a direct antagonism of histamine was also involved.

Aaron and Abramson[1] introduced Pyribenzamine and Procaine into human skin by iontophoresis one and one-half hours before introducing histamine. Pyribenzamine inhibited the histamine wheal and flare reaction, and, since Procaine failed to do so, one can assume that the positive results with Pyribenzamine were referable to an antagonism of histamine and not to a local anesthetic action, unless it is shown that such action is exceedingly prolonged. These experiments with Benadryl and Pyribenzamine certainly emphasize the necessity of considering local anesthetic action when interpreting the influence of antihistamine drugs on cutaneous responses, especially when drugs are applied topically or injected locally. The author is not aware of any direct evidence that systemic anesthetic action is induced by oral administration of moderate quantities of antihistamine drugs. However, oral doses frequently diminish cutaneous reactions to histamine (see Loew,[33] page 558). The evidence cited enhances the probability that a direct antagonism of histamine accounts for the fact that antihistamine drugs diminish cutaneous responses to histamine.

Other evidence eliminates the possibility that an atropine-like action of antihistamine drugs accounts for the alteration of cutaneous responses and also provides support for belief in antihistamine specificity. In rabbits, Last and Loew[25] measured the degree of increased capillary permeability in the abdominal skin by observing the extent to which intravenously injected Trypan Blue dye extravasated into areas receiving intradermal injections of histamine and other substances. Positive Trypan Blue responses, indicative of increased capillary permeability, were readily induced with histamine. Pretreatment of the animals with Benadryl or Neoantergan, by parenteral administration, diminished or eliminated the increased capillary permeability due to histamine as indicated by diminished or abolished Trypan Blue responses. The increased capillary permeability induced with trypsin, snake venom, staphylococcus toxin, tetracaine (Pontocaine), and codeine was not altered by treatment with Benadryl, or by Neoantergan when the latter was used. The failure of antihistamine drugs to alter responses to these substances suggests that liberated histamine did not cause the increased capillary permeability. Prevention of responses to histamine could scarcely be due to any atropine-like action of antihistamine drugs, since Neoantergan was very effective even though nearly devoid of atropine-like action. Furthermore, atropine itself does not alter this type of cutaneous reaction in man.[56] Because of its antiacetylcholine specificity, one would not expect it to alter cutaneous responses to histamine, since it is improbable that any choline esters are involved in the reaction. Neither Mecholyl[25] nor acetylcholine[48] induced Trypan Blue reactions in rabbits.

If the ability of antihistamine drugs to diminish Tyrpan Blue reactions to histamine were related to vasodilator or vasoconstrictor actions of the drugs, the responses to the other substances should have been altered also. Added consideration must be given to the possibility that vasoconstriction aids in diminishing the Trypan Blue response to histamine because of the previously mentioned fact that several antihistamine drugs enhance the pressor (vasoconstrictor?) effect of epinephrine. We have repeated the experiments in rabbits[26] and determined that the Trypan Blue responses to histamine were readily annulled by α-naphthylmethylethyl-β-chloroethylamine in doses which were known to be adequate to diminish several effects of histamine in other animal species and which blocked the pressor action of epinephrine in rabbits. It was noted also in these experiments that the naphthyl derivative greatly diminished the blanching due to vasoconstriction in areas injected intradermally with epinephrine. Thus, the ability of histamine to increase capillary permeability was annulled by a drug which antagonized histamine and the vasoconstrictive action of epinephrine. This evidence, in conjunction with other considerations, supports the thesis that vasoconstriction does not account for the ability of antihistamine drugs to prevent histamine from increasing capillary permeability.

Thus, an analysis of the experimental evidence cited, as well as a review of other pertinent information,[33] leads to the belief that the ability of antihistamine drugs to diminish the cutaneous flare and wheal response to histamine is largely due to a specific blocking of the action of histamine. Histamine-induced vasodilatation and increased capillary permeability are diminished, and these effects cannot be due entirely to local anesthetic, atropine-like, or vasoconstrictive actions of antihistamine drugs. The local anesthetic action of several antihistamine drugs is sufficiently strong to diminish cutaneous reactions to histamine and probably to other agents, at least when the drugs are applied topically or injected locally.

Discussion

Analysis of the available evidence reveals that antihistamine drugs such as Antergan, Antistine, Neoantergan, Benadryl, Pyribenzamine, and the thiophene analogue of the latter are capable of diminishing the ability of histamine to contract smooth muscle of several organs, to relax vascular smooth muscle, and to increase permeability of capillary endothelium. The gastric secretogogue action of histamine is not blocked, and there are insufficient data to prove that the secretogogue action on other cells is affected.

The prominence of the drug antagonism of the contracting and relaxing effects of histamine on smooth muscle and the increase of capillary permeability suggest that a relatively specific antihistamine action is involved. Other pharmacological actions have been demonstrated with antihistamine drugs, but the foregoing analysis reveals that experiments of proper design yield evidence which indicates that none of these actions can account for any more than a small part of histamine antagonism. The evidence supports the thesis that the drugs discussed are relatively specific antihistamine drugs or antihistaminics. In order to avoid confusion in this rapidly developing phase of pharmacology, it is recommended that new drugs should not be classified as antihistaminics unless a moderate degree of antihistamine specificity has been demonstrated.

Several β-chloroethylamine derivatives, chemically related to Dibenamine,[45] have been demonstrated to be moderately or highly effective in diminishing several effects of histamine.[2, 33, 39] At present, however, the author is reluctant to class these compounds as antihistaminics in view of their marked ability to induce epinephrine reversal and to diminish, block, and reverse some responses to adrenergic nerve stimulation. Parenthetically, the author wishes to admit that confusion may stem from the statement that higher alkyl homologues of the β-chloroethylamines recently studied were devoid of histamine antagonistic properties.[2, 33] In contrast to lower alkyl homologues, they did fail to prevent histamine-induced bronchioconstriction in guinea pigs, but recent experiments indicate that all the compounds, including the higher alkyl homologues, are capable of diminishing and blocking the depressor effects of histamine in anesthetized dogs.[34] Extension of these studies may establish the fact that certain compounds can preferentially select a given tissue and antagonize the effects of histamine thereon. Recognition of this possibility should suffice to warn that a single type of pharmacological test might fail to reveal an ability to antagonize some effect of histamine.

The very variety of pharmacological effects which is elicited to some degree by antihistamine drugs investigated thus far suggests that even other types of action may yet be demonstrated and that future antihistamine drugs may possess various combinations of pharmacological properties. In fact, the demonstration of histamine antagonism possibly should be regarded as evidence that the drugs in question have some special affinity for certain components of effector cells. Slight qualitative differences between the natures of such affinities may determine whether the drugs exert prominent antihistamine action or some other blocking or stimulating action on muscle, nerve, secretory, or endothelial cells.

The varied but somewhat minor pharmacological actions of antihistamine drugs which detract from the degree of specificity may be of more importance than realized, since they could conceivably throw light on the locus and mode of action of these drugs. It may not be mere coincidence, for example, that the several chemical types of drugs which antagonize histamine all either enhance or diminish certain effects of epinephrine, and that all exert some antiacetylcholine and local anesthetic action. These varied actions may be dependent upon some common mechanism or several closely related ones.

Summary

(1) Antihistamine drugs diminish or block the most prominent actions of histamine, except the gastric secretogogue action. Data relating to other secretory effects are needed.

(2) The drugs exert a relatively high degree of specific antihistamine action on smooth muscle and capillaries, since, under proper conditions, this action is readily demonstrable with small doses and is usually nondependent on local anesthetic, antispasmodic, spasmogenic, sympathomimetic, or sympatholytic action.

(3) It is very unlikely that antihistamine action on any tissue is referable to either an enhancement or diminution of the excitatory effects of epinephrine, since several potent histamine antagonists enhance the pressor response to epinephrine, whereas others strongly antagonize histamine although they diminish and even block and reverse the pressor effects of epinephrine.

(4) When antihistamine drugs, even though relatively specific, are used as research tools or diagnostic aids, due consideration must be given to a variety of pharmacological actions when experiments are planned and the findings are interpreted. For example, high local concentration of these drugs following topical application or local injection can induce a degree of local anesthesia capable of diminishing cutaneous responses to histamine and probably to other agents.

(5) When measured under certain conditions, the potency of Benadryl is less than that of Neoantergan and Pyribenzamine. The antihistamine specificity of Benadryl may therefore be less, but consideration must also be given to the fact that, in some circumstances, no differences in potency have been demonstrated. Furthermore, each of these compounds enhances the pressor action of epinephrine, and the antispasmodic action of Benadryl is replaced by a weak spasmogenic action in all the ethylenediamine derivatives. There has been no demonstration of a pronounced variation in the degree of local anesthetic action induced by antihistamine drugs.

Addendum

A majority of the side effects in human beings by antihistaminics are probably unrelated to a specific histamine blocking action. Furthermore, more recent evidence of the effectiveness of certain antihistaminics in controlling symptoms of motion sickness and Parkinsonism suggests drug activities other than blockade of histamine. It is probable that future studies with compounds now regarded as highly specific antihistaminics will reveal other nonspecific actions of theoretical and practical interest.

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ANTISTINE AND RELATED IMIDAZOLINES

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The series of aromatic imidazolines which have been investigated for about ten years in our laboratories has offered special possibilities for the systematic variation of pharmacological effects. This is due to the fact that, in this group, substances are found with a specific effect in several directions.

The histaminolytic efficiency of numbers of the aromatic imidazolines is only an example of this specificity of effect. One of the most effective substances in this direction is Antistine[1] (formula, see page 1163). Its structural formula is similar to that of Antergan. A series of imidazoline derivatives possess, likewise, a high histaminolytic effect, though small changes in the structure are sufficient to reduce strongly this efficacy.

Antistine shows, in comparison with the other histaminolytics, several special properties. The toxicity of all histaminolytically effective substances is about of the same order. However, the dose with which the first side-effects are observable is different. According to our experience, this dose is relatively higher in the case of Antistine than for other substances. Also, the tolerability on local application seems to be better. Antistine, as an antihistaminic agent, is less active than Pyribenzamine,[2] Neoantergan,[3] and the Phenothiazine[4] derivatives. This is as true for the reaction of isolated organs as for the antagonistic effect on histamine toxicity in the animal. The difference varies, depending on the object. On the other hand, the action on the anaphylactic shock is not less but is of the same order of potency as that of the most effective antihistaminics (TABLE 1). Besides these effects, Antistine differs from the other antihistaminics in its non-antihistaminic pharmacological actions. For example, it has no pronounced antiacetylcholine nor adrenaline reinforcing effect; on the contrary, it has a slight sympathicolytic action on different test objects (FIGURE 1).

These differences show that Antistine possesses a complex of pharmacological effects of its own. They show further that pronounced antianaphylactic activity may be produced without any marked effect on the autonomic system (as has been demonstrated already by Loew). On the other hand, the antihistaminic effect seems to be correlated, in many different chemical structures, with the effects on the autonomic nervous system. This seems to indicate that those chemical compounds potentially possess the other specific features.

It seemed to be interesting from the theoretical and practical point of view to investigate whether substances could be devised from the same basic structure, possessing only the one or the other of those specific effects. In the series of aromatic imidazolines synthesized by Hartmann and Isler and Miescher, Urech, and Klarer, we established examples to elucidate this possibility. In the series of benzimidazolines, it was already striking that, by small changes of the molecule, strongly effective sympathicomimetics could be converted into sympathicolytics (see FIGURE 2.)

Here, it is perhaps noteworthy that the sympathicomimetic, 2020/n, and some other similar substances of this series have approximately a ten to one hundred times stronger sympathicomimetic effect on the vessels and on the blood pressure than epinephrine or norepinephrine. In view of the characterization of the sympathicomimetic receptors, this fact has a certain interest.[10]

TABLE 1

COMPARISON OF ANTIHISTAMINIC AND ANTIANAPHYLACTIC ACTION OF ANTIHISTAMINIC DRUGS ON DIFFERENT TEST OBJECTS

Preparations	*Toxicity Rabbit i.v. (L.D. 50)*	*Spasmolysis histamine-test Guinea-pig* (5×10^{-8})	*Protection in vitro Schultz-Dale*	*Protection in vivo Guinea-pig against anaphyl death*	*Number of lethal histamin-doses i.v. Guinea-pig (20 mg./kg. cc.)*
Antergan	2×10^{-5}	2.5×10^{-8}	2.5×10^{-8}	5×10^{-7}	120
Neo-antergan	1.9×10^{-5}	2×10^{-9}	10^{-8}	5×10^{-6}	300
Pyribenzamine	1.4×10^{-5}	2×10^{-8}	2×10^{-8}	3×10^{-6}	150
Antistine	1.8×10^{-5}	10^{-7}	10^{-7}	3×10^{-6}	30
Phenothiazine JI 3277 R.P.	·/.	5×10^{-8}	·/.	10^{-6}	1500

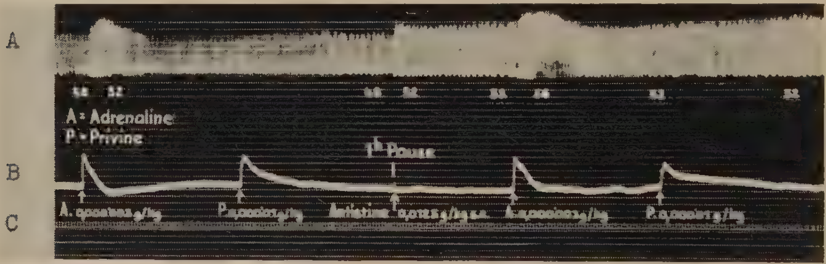


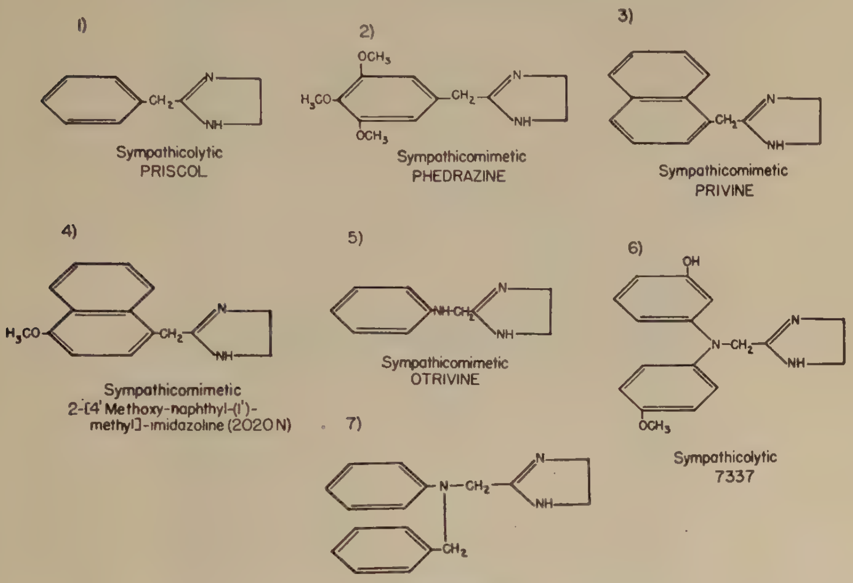
FIGURE 1. Comparison of the pressor effect of adrenaline and Privine, before and after s.c. Antistine treatment. No adrenaline reinforcing effect. A—Respiration; B—Blood pressure; C—Time 6″.

Still greater than the series of benzylimidazolines are the possibilities of variation in the series of aromatic imidazoline derivatives of aniline. Antistine belongs to this series. Also, strongly effective sympathicomimetics such as Otrivine have been found. Only lately, one of the strongest effective sympathicolytics has been detected in this series.

The basic structure in this series shows a clearly defined differentiation which evidently determines the change in specific effect. Nuclear substitutes can influence, in more or less every case, the intensity of the specific efficiency. This shows that, in this series, compounds, in spite of great chemical similarity, react in a fundamentally different way with the cell substrate.

For the characterization of the specificity of effect, the question is raised, first of all, whether this specificity is complete in every case. For the sympathicolytic and antihistaminic action, the comparison with a physiological humoral factor of the body does not permit a decision of this question. Only for the sympathicomimetic effect does a comparison with the physiological agent, epinephrine or norepinephrine, give this information.

The classification of the effect according to the nature and intensity, for a series of different objects, shows that the sympathicomimetic imidazolines act analogously to adrenaline upon a whole series, but not on others.



1) Sympathicolytic PRISCOL

2) Sympathicomimetic PHEDRAZINE

3) Sympathicomimetic PRIVINE

4) Sympathicomimetic 2-[4' Methoxy-naphthyl-(1')-methyl]-imidazoline (2020 N)

5) Sympathicomimetic OTRIVINE

6) Sympathicolytic 7337

7) Antihistaminic ANTISTINE

FIGURE 2.

The vasoconstrictive effect of Otrivine and also of Privine on the isolated vessels and on blood pressure is of the same order as that of epinephrine (FIGURE 3). On the isolated heart, the coronary dilatation of adrenaline is missing just as is the effect on amplitude and frequency. Therefore, a different action on the circulation, especially the perfusion of the different peripheral areas, occurs as previously published.[8]

The effect on the isolated seminal vesicles and the isolated intestine of the rabbit is similar to that of adrenaline—stimulating in the first and relaxing in the latter. On the isolated intestine of the guinea pig, the action of the imidazoline is contrary to that of adrenaline. This sympathicomimetic effect consequently favors only a selected number of sympathetic points of attack.

A characterization of the sympathicolytic and histaminolytic effects of imidazolines can result from their comparison with other analogous substances or investigations of the antagonistic effect towards histamine, adrenaline, acetylcholine, *etc.*

The comparison of the effects of sympathicolytic and histaminolytic imidazoline derivatives with those of the primary effective substances, such as adrenaline and histamine, shows that there is, in general, a typical contrary action on most of the objects, namely, on the blood pressure, on the intestine, and on the seminal vesicles. On isolated objects, however, a similar effect occurs exceptionally, independent of whether a sympathicolytic or a histaminolytic effect is also present. These differences may be attributed partly to the different physiological autonomic equilibrium of the organs tested but could not be interpreted generally in this way.

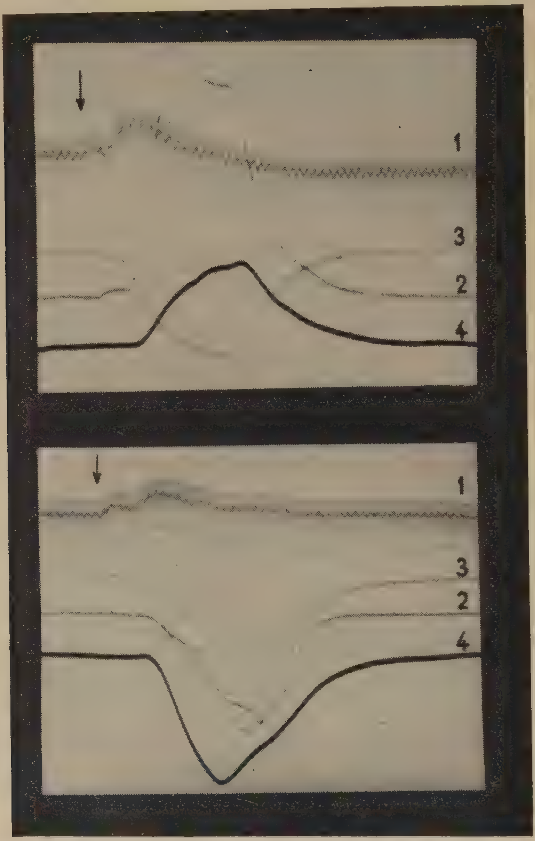
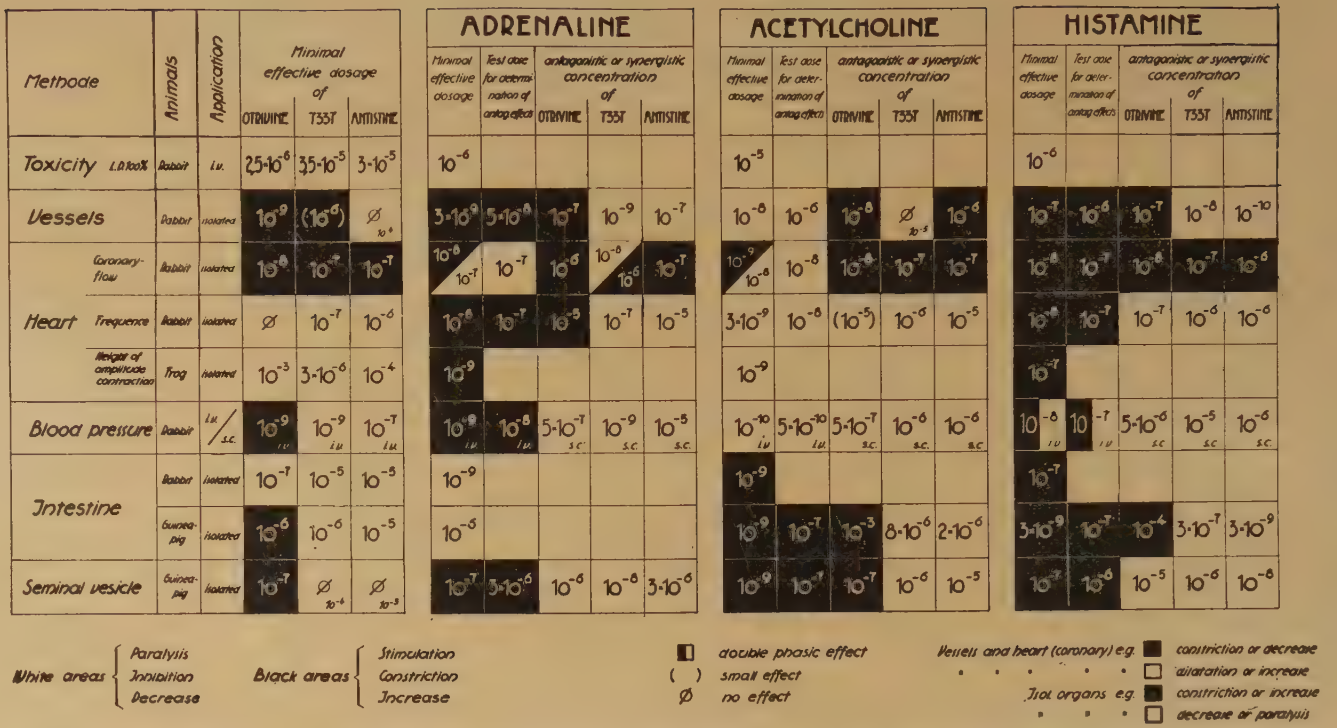


FIGURE 3. Effect of adrenaline and Privine on the blood pressure and on the perfusion of different arterial areas on the cat. 1—blood pressure of the carotid artery; 2—perfusion of the mesenteric artery; 3—perfusion of the renal artery; 4—perfusion of the femoral artery. A—adrenaline 2γ/kg. i.v.; B—Privine 5γ/kg. i.v. Change of records of arterial perfusion: upwards—increase; downwards—decrease.

The specific antagonistic effect of those drugs has to be clarified by testing the antagonistic action on different organs. The results of this investigation indicate that, in each group of imidazolines with specific effect, a series of antagonisms of high specificity could be demonstrated (FIGURE 4).



Methode		Animals	Application	Minimal effective dosage of			ADRENALINE					ACETYLCHOLINE					HISTAMINE				
							Minimal effective dosage	Test dose for determination of antag. effects	antagonistic or synergistic concentration of			Minimal effective dosage	Test dose for determination of antag. effects	antagonistic or synergistic concentration of			Minimal effective dosage	Test dose for determination of antag. effects	antagonistic or synergistic concentration of		
				OTRIVINE	T33T	ANTISTINE			OTRIVINE	T33T	ANTISTINE			OTRIVINE	T33T	ANTISTINE			OTRIVINE	T33T	ANTISTINE
Toxicity	L.D.100%	Rabbit	i.v.	$2{,}5 \cdot 10^{-6}$	$3{,}5 \cdot 10^{-5}$	$3 \cdot 10^{-5}$	10^{-6}					10^{-5}					10^{-6}				
Vessels		Rabbit	isolated	■ 10^{-9}	■ (10^{-6})	Ø 10^{-4}	■ $3 \cdot 10^{-9}$	■ $5 \cdot 10^{-8}$	■ 10^{-7}	10^{-9}	10^{-7}	10^{-8}	10^{-6}	■ 10^{-8}	Ø 10^{-5}	■ 10^{-6}	■ 10^{-7}	■ 10^{-6}	■ 10^{-7}	10^{-8}	10^{-10}
Heart	Coronary-flow	Rabbit	isolated	■ 10^{-8}	■ 10^{-7}	■ 10^{-7}	■ 10^{-8} / 10^{-7}	10^{-7}	■ 10^{-6}	10^{-8} / ■ 10^{-6}	■ 10^{-7}	■ 10^{-9} / 10^{-8}	10^{-8}	■ 10^{-8}	■ 10^{-7}	■ 10^{-7}	■ 10^{-8}	■ 10^{-7}	■ 10^{-8}	■ 10^{-7}	■ 10^{-6}
	Frequence	Rabbit	isolated	Ø	10^{-7}	10^{-6}	■ 10^{-8}	■ 10^{-7}	■ 10^{-5}	10^{-7}	10^{-5}	$3 \cdot 10^{-9}$	10^{-8}	(10^{-5})	10^{-6}	10^{-5}	■ 10^{-8}	■ 10^{-7}	10^{-7}	10^{-6}	10^{-6}
	Height of amplitude contraction	Frog	isolated	10^{-3}	$3 \cdot 10^{-6}$	10^{-4}	■ 10^{-9}					10^{-9}					■ 10^{-7}				
Blood pressure		Rabbit	i.v. / s.c.	■ 10^{-9} i.v.	10^{-9} i.v.	10^{-7} i.v.	■ 10^{-9} i.v.	■ 10^{-8} i.v.	$5 \cdot 10^{-7}$ s.c.	10^{-9} s.c.	10^{-5} s.c.	10^{-10} i.v.	$5 \cdot 10^{-10}$ i.v.	$5 \cdot 10^{-7}$ s.c.	10^{-6} s.c.	10^{-6} s.c.	◧ 10^{-8} i.v.	◧ 10^{-7} i.v.	$5 \cdot 10^{-6}$ s.c.	10^{-5} s.c.	10^{-6} s.c.
Intestine		Rabbit	isolated	10^{-7}	10^{-5}	10^{-5}	10^{-9}					■ 10^{-9}					■ 10^{-7}				
		Guinea-pig	isolated	■ 10^{-6}	10^{-6}	10^{-5}	10^{-5}					■ 10^{-9}	■ 10^{-7}	■ 10^{-3}	$8 \cdot 10^{-6}$	$2 \cdot 10^{-6}$	■ $3 \cdot 10^{-9}$	■ 10^{-7}	■ 10^{-4}	$3 \cdot 10^{-7}$	$3 \cdot 10^{-9}$
Seminal vesicle		Guinea-pig	isolated	■ 10^{-7}	Ø 10^{-4}	Ø 10^{-3}	■ 10^{-7}	■ $5 \cdot 10^{-6}$	10^{-6}	10^{-8}	$3 \cdot 10^{-6}$	■ 10^{-9}	■ 10^{-7}	■ 10^{-7}	10^{-6}	10^{-5}	■ 10^{-7}	■ 10^{-6}	10^{-5}	10^{-6}	10^{-8}

White areas { Paralysis, Inhibition, Decrease

Black areas { Stimulation, Constriction, Increase

◧ double phasic effect

( ) small effect

Ø no effect

Vessels and heart (coronary) e.g. ■ constriction or decrease

. . . . . . □ dilatation or increase

Isol. organs e.g. ■ constriction or increase

. . . □ decrease or paralysis

FIGURE 4. Comparison of action of different imidazoline derivatives with the effect of adrenaline, acetylcholine, and histamine and of the antagonistic effect to these substances.

The examination, particularly the quantitative evaluation of antagonistic reactions, presents various difficulties. They consist of the special conditions which have to be ascertained according to the relative dose and the size of the dose evaluated for each test organ. Another difficulty is the fact that, on certain organs, no antagonistic effects occur. Thus, the possibilities of evaluation are limited in many respects.

If a typically suitable test object is chosen (*e.g.*, the reaction of the isolated vessels), the relatively high specificity of the antagonistic reaction can be easily demonstrated. For example, the antagonism of the sympathicolytic for adrenaline is in proportion to Antistine and Otrivine in the ratio of 100:1:0, or the antagonism of Antistine for histamine is in proportion to both the others as 100:1:0; the relations are the opposite in these cases. As to the reaction on the seminal vesicles, the specificity is present in the same way. The relative proportion of effect is somewhat different; thus, for example, adrenaline antagonism, sympathicolytic 7337: Otrivine: Antistine = 100:3:1 and the histamine antagonism of Antistine:sympathicolytic 7337:Otrivine = 100:1:0.1.

Nearly the same relation exists for the reaction of the isolated guinea-pig intestine. On other objects, the relative effectiveness of the two compounds shows other differences. The histamine effect on the isolated heart is counteracted by both substances in the same concentration. Regarding blood pressure, Antistine does not alter the adrenaline effect. The sympathicolytic acts in the same antagonistic concentration as on other organs. These few examples show that, again, the antagonistic effect, specific in principle, is typical only on certain organ systems.

The investigation of the antagonistic action against acetylcholine demonstrates that none of these substances has a specific antagonistic effect but that occasionally such may be present. The isolated vessels, dilated by acetylcholine, are constricted by Antistine, in spite of the fact that it has no action by itself. The sympathicolytic agent has no effect even in high concentrations. Both substances act antagonistically to the same degree on the seminal vesicles.

These differences of antagonism are independent of the primary effect upon the normal function, which exists without the previous influence of the agonists. This shows that the specific effect of the antagonists appears only when a potential action of the agonists exists. Consequently, these antagonistic substances will exert their specific action only when a specific primary effect is present; otherwise, their "unspecific" effects will appear. In principal, these belong in the same class as the primary effects or they are unspecifically antagonistic.

The demonstration of an effect of a high degree of specificity on individual objects in no way excludes the absence of this effect or the presence of unspecific actions on other objects. The dosage range between these specific and nonspecific effects is not always large enough to exclude the fact that these unspecific effects are not involved in the therapeutic phenomenon or the side reactions.

These results show that the group of aromatic imidazolines contains substances with high specificity, but they show also that every group has a specific type of action when compared with other members of the same pharmacological group. This can be shown for the sympathicolytic effect in a way similar to that done with the sympathicomimetic and antihistaminic effect (*i.e.*, by comparison with other sympathicolytic agents), as we have already published. The analysis of the circulatory effect is especially suitable. Of these substances, only one example need be given for the group of sympathicolytics. In spite of the two substances, Priscol and 7337, producing an adrenalin reversal on the blood pressure, the circulatory response measured on the perfusion of different arterial areas as mesenteric, femoral, and renal artery shows reversal of blood flow reaction only in the case of Priscol—not with 7337 (FIGURE 5). So it can be seen that, in spite of typical effect in one direction, the same may be lacking in another one. Thus, most groups of specific agents seems to have an individual type of effect.

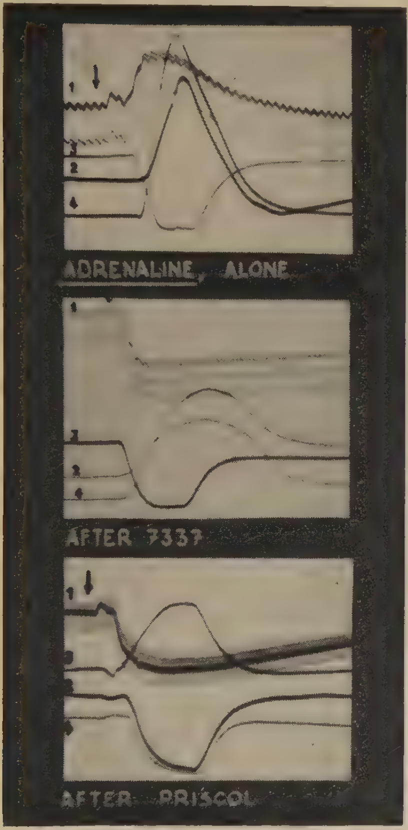


FIGURE 5. Effect of pretreatment with Priscol and sympathicolytic imidazoline 7337 on the adrenaline effect on blood pressure and the perfusion of different arterial areas on the cat. 1—blood pressure of the carotid artery; 2—perfusion of the mesenteric artery; 3—perfusion of the renal artery; 4—perfusion of the femoral artery. A—adrenaline 2γ/kg. i.v.; B—adrenaline 2γ/kg. i.v. after pretreatment with 0.010 g./kg. s.c. 7337; C—adrenaline 2γ/kg. i.v. after pretreatment with 0.025 g./kg. s.c. Priscol. Change of records of arterial perfusion: upwards = increase; downwards = decrease.

Since, in the group of the aromatic imidazolines with fundamentally similar chemical structure, both a pronounced system-restricted and an organ-restricted differentiation of various specific effects occur as a result of small chemical changes, it is permissible to conclude that the influenced substrate must possess an analogous differentiation. It is responsible both for the systematic effect of the agonists and antagonists and the multiplicity of the possibilities of differentiation. This differentiation must be of such a type that the system can react upon substances of a quite different nature with contrary or differentiated reaction, and that, in the case of a large degree of chemical similarity of the substances, completely divergent reactions can arise.

Under these circumstances, it seemed to be of special interest to investigate whether differences in the behavior of these imidazolines could be found which are parallel to their specific effectiveness. The quantitative pharmacological analysis of the reaction with the substrate has been investigated in different directions and, I may add, without great success. In spite of that, however, the results of these investigations seem to have some general importance.

The determination of the dose-effect curves gives the reaction equilibrium. In this respect, the dose-effect curve of the primary effective substances and of the antagonistic reactions are to be considered. These should be determined on the greatest possible number of objects, where a specific or more unspecific effect is present. Various difficulties stand in the way of such investigations, which consist partly in the great number of necessary experiments, partly in the primary and antagonistic reactions not occurring in the same way, *etc.* The dose-effect curves for the primary substances—adrenaline, histamine, acetylcholine, and other substances—were ascertained, and the antagonistic effect of specifically acting imidazolines and of other selected substances towards the primary effects were likewise established. For example, we have investigated as follows:[12,13,14] on the seminal vesicles—adrenaline, acetylcholine, histamine, and Otrivine; on the vessels—a greater number of sympathicomimetics and acetylcholine; on the intestine—acetylcholine, histamine, Priscol, and Otrivine. All the substances tested on the same object give very similar dose-effect curves (FIGURE 6). Moreover, it is a matter of indifference whether the substances vary in efficacy by several powers of 10 or belong to quite different groups. Only occasional differences exist between certain dose-effect curves. These, perhaps, have a decisive importance. Considering the margin of error of our methods, however, it is premature to draw any conclusions.

This can signify that the specific, different substrates for the individual substances react in a very similar way towards the various substances or that an unspecific physico-chemical process of the equilibrium solution/cell determines the analogous behavior, and that the characteristic properties of the specific point of attack is concealed by these. The dose-effect curve is consequently not to be made directly responsible for the specificity of the effect. In most cases, the difference of the reaction consists exclusively in the variation of the absolute concentration present in the solution which is necessary for the production of equal effects.

The antagonistic effects have to be investigated in the same way. In general, there exists a parallelism of the antagonistic dose-effect curves and of those of the primary effect, inasmuch as the former have, largely, a somewhat flatter course. The dosage-action curves of the specific sympathicolytic agents against adrenaline and a sympathicomimetic imidazoline

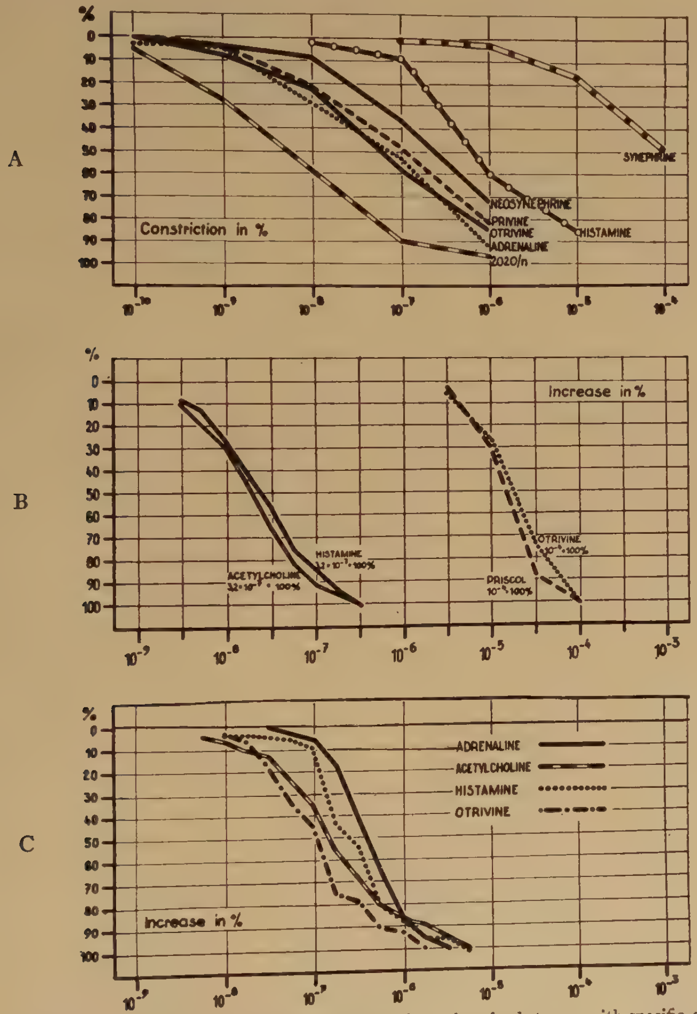


FIGURE 6. Dose-effect graphs showing the action of a series of substances with specific and nonspecific effect on perfused isolated blood vessels of the rabbit and the seminal vesicle and the isolated intestine of the guinea pig. A—action on perfused hindlegs of the rabbit (abs.—concentration of drug; ord.—inhibition of flow in percentage); B—action on isolated intestine of the guinea pig (abs.—concentration of drug; ord.—contraction in percentage of the maximal response); C—action on isolated seminal vesicle of the guinea pig (abs.—concentration of drug; ord.—contraction in percentage of the maximal response).

are all parallel, but the "unspecific action" of acetylcholine also shows the same behavior (FIGURE 7).

On the isolated intestine only the antagonistic effect on acetylcholine and histamine can be determined. The curves of the antagonistic effect of Antistine, atropine, and adrenaline to histamine have a similar shape; the sympathicolytic imidazoline is different (FIGURE 8). The antagonistic reaction of atropine, Antistine, and the sympathicolytic imidazoline against

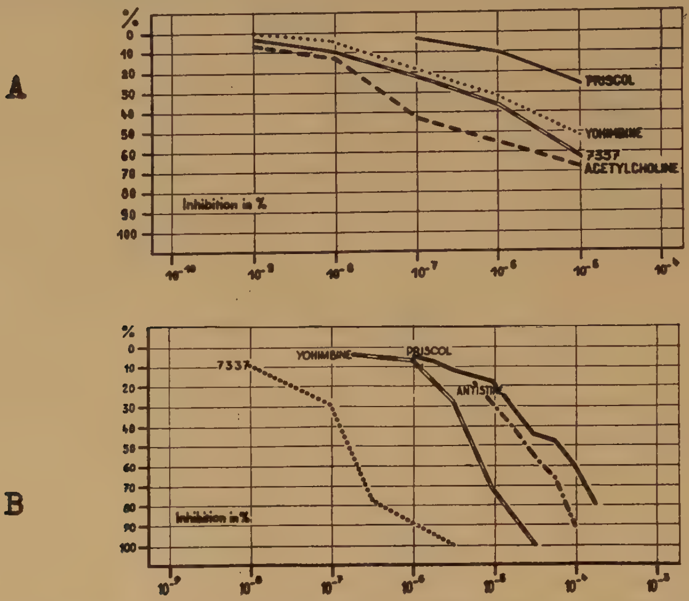


FIGURE 7. Dose-effect graphs showing antagonistic effect of different drugs to adrenaline on different test objects. A—antagonistic effect on isolated perfused hindleg of the rabbit (inhibition of adrenaline contraction—50–60 per cent of maximal response: abs.—concentration of drug; ord.—inhibition of adrenaline constriction in per cent of flow); B—antagonistic effect on isolated seminal vesicles of the guinea pig (inhibition of maximal response with threshold dose of adrenaline in per cent of maximal adrenaline effect: abs.—concentration of drug; ord.—inhibition of adrenaline contraction in percentage).

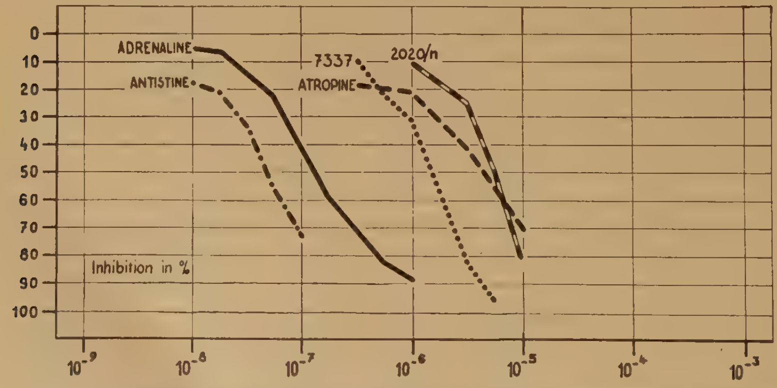


FIGURE 8. Dose-effect graph showing antagonistic effect of different drugs to histamine on the isolated intestine of the guinea pig. Inhibition of maximal response with threshold dose of histamine in per cent of maximal histamine effect: abs.—concentration of drug; ord.—inhibition of histamine contraction in percentage.

acetylcholine is similar; only the adrenaline curve shows a different shape (FIGURE 9). The reaction on the seminal vesicle also shows no certain difference between the curve of the specific sympathicolytic and other unspecific substances against adrenaline. Certainly these examples are only a few out of the number possible and perhaps necessary. In general, however, the results may justify the assumption that there exists no simple connection between the dosage-effect curve and the specificity of the antagonistic effect.

A second possibility for the characterization of the type of reaction consists in the determination of the time-effect relation. If there are definite differences in the time where maximal effects are produced, these could also be responsible for the strength of the effect.

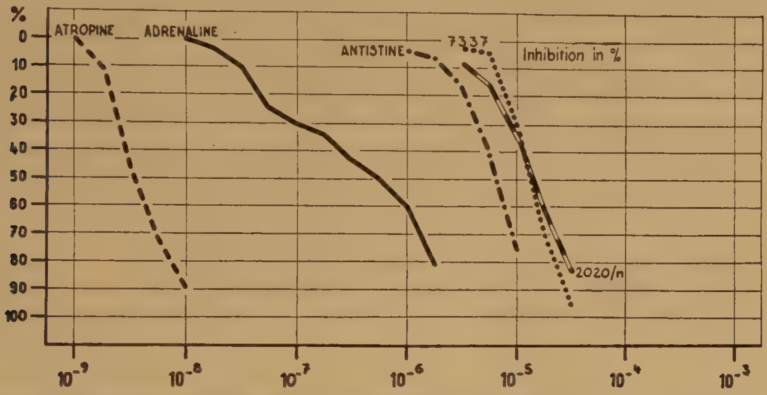


FIGURE 9. Dose-effect graph showing the antagonistic effect of different drugs to acetylcholine on the isolated intestine of the guinea pig. Inhibition of maximal response with threshold dose of acetylcholine in per cent of maximal acetylcholine effect: abs.—concentration of drug; ord.—inhibition of acetylcholine contraction in percentage.

In such experiments, the penetration velocity naturally plays a significant part. The penetration velocity is likewise a characteristic of the reaction of the various substances which have importance for the specific equilibrium, *etc.*, without being necessarily of direct importance to the specific effect. Technically, the antagonist is added to the test object and subsequently the agonistic compound at various intervals (FIGURES 10 and 11). In all the experiments, concentrations were chosen so that the agonistic substance produced a full contraction, while the antagonist abolished this by approximately 60–70 per cent. Despite the fact that it resulted in a series of differences, the latter can give no explanation of the specific effect of the imidazolines. Although, in individual tests, the maximal effect for the specific antagonists is reached sooner than that for the unspecific effective imidazolines, the differences are small, and even the unspecific atropine effect, *e.g.*, in the histamine antagonism, attains the maximum somewhat more quickly.

A further possibility for characterizing the behavior of the reaction consists in the inhibitory effect on systems being investigated about which a specific chemical reaction with the primary stimulatory substances is known, *i.e.*, for example, on enzymes which destroy the latter. It should be stressed that these investigations have not the intention of bringing directly together

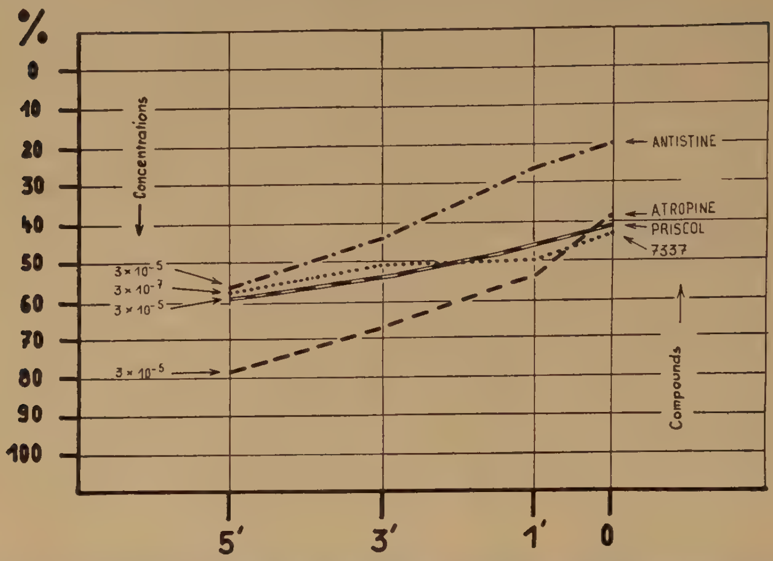


FIGURE 10. Development of antagonistic effect of different drugs to adrenaline with interval between addition of these substances. Abs.—time after application of drug before addition of 3.2 × 10^{-6} of adrenaline; ord.—inhibitory effect in percentage of response (effect on 5′ corresponds to maximal antagonistic effect).

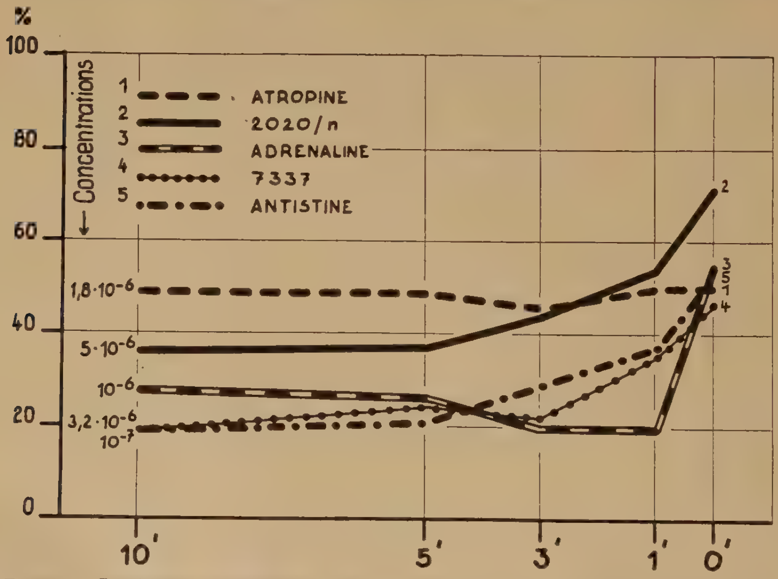


FIGURE 11. Development of antagonistic effect of different drugs to histamine with interval between addition of these substances. Abs.—time after application of drug before addition of 5 × 10^{-8} of histamine; ord.—inhibitory effect in percentage of response (effect on 10′ corresponds to maximal antagonisic effect).

the point of attack with this ferment mechanism, but rather of characterizing the behavior in comparison to the reaction with the specific cell receptors. With this purpose, the inhibitory effect towards diamine oxidase, serum cholinesterase, and adrenaline oxidase was investigated by Schuler in our laboratory. It was found that, in the effect upon diamine oxidase, there exist such small differences between the specific histamine antagonists and other substances that the specific antagonism to histamine on the cell substrate must have a different character from the enzymatic reaction (FIGURE 12).

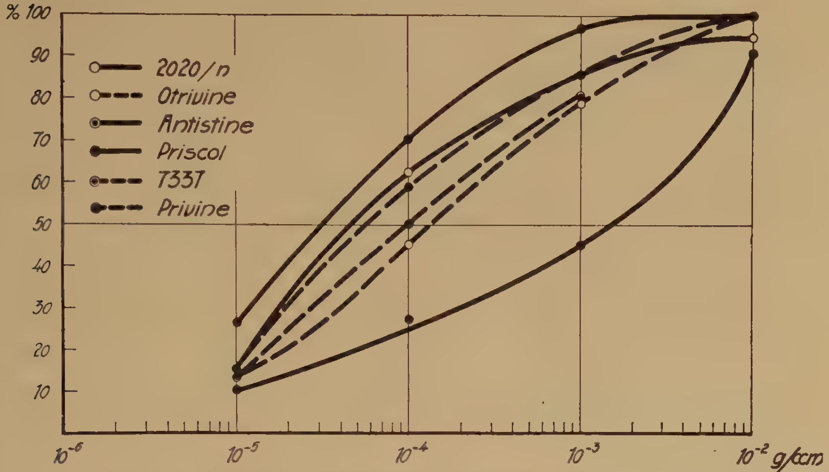


FIGURE 12. Inhibition of the action of diamineoxidase (histaminase), produced by imidazoline derivatives with different specificity of effect: Privine, Otrivine, 2020/n—sympathicomimetic; Priscol, 7337—sympathicolytic; Antistine—antihistaminic. Abs.—concentration of drug; ord.—inhibition in per cent of control after 2 hours.

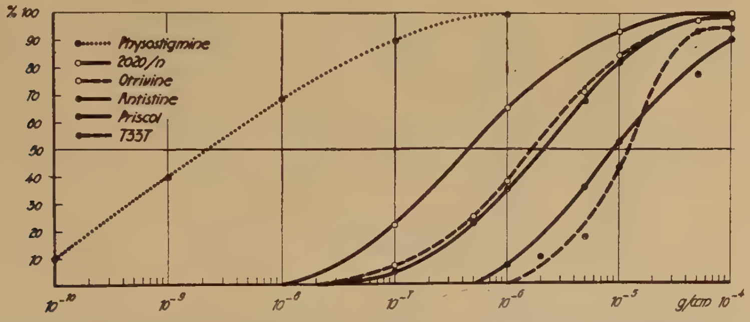


FIGURE 13. Inhibition of the action of serum cholinesterase produced by imidazoline derivatives with different specificity of effect in comparison to Eserine: Otrivine, 2020/n—sympathicomimetic; Priscol, 7337—sympathicolytic; Antistine—antihistaminic. Abs.—concentration of drug; ord.—inhibition in per cent of control after 2 hours.

The situation is somewhat different in the case of serum cholinesterase, where greater differences exist. In particular, the effectiveness of some of the sympathicomimetic imidazolines in inhibition of the cholinesterase is relatively pronounced (FIGURE 13). There are in the whole animal, however, no signs of acetylcholine-like effects that could not be in connection with the specific action.

As adrenaline oxidizing agents, a highly active polyphenoloxidase and an amino oxidase have been investigated. The effects on the polyphenoloxidase are uncharacteristic (FIGURES 14 and 15). On the amino oxidase, the sympathicomimetic imidazolines prove the most effective and in the same order as their relative sympathicomimetic effectiveness in the animal. But the necessary concentrations are much higher for the enzyme inhibition than for the action on pharmacological test objects. On the other hand, the effect of sub-threshold dosages of adrenaline are not reinforced by these products. These results seem only to indicate that there is some similarity in reaction between the aminophenoloxidase and the pharmacological reactive system.

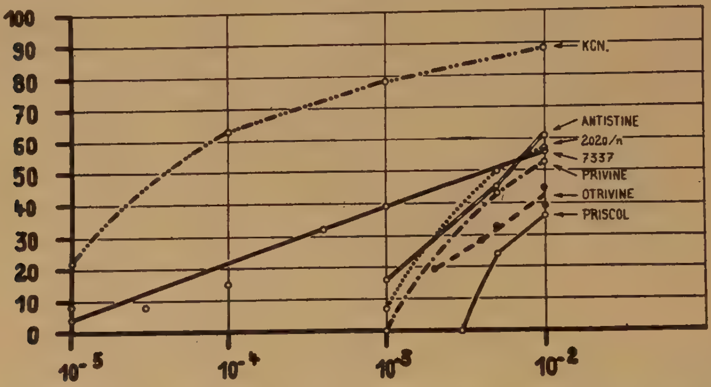


FIGURE 14. Inhibition of the action of Polyphenoloxidase on adrenaline produced by imidazoline derivatives with different specificity of effect in comparison to K.C.N.: Privine, Otrivine, 2020/n—sympathicomimetic; Priscol, 7337—sympathicolytic; Antistine—antihistaminic. Abs.—concentration of drug; ord.—inhibition in per cent of control after 2 hours.

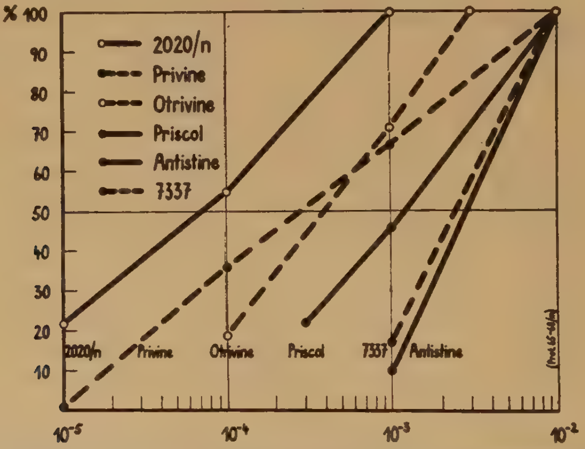


FIGURE 15. Inhibition of the action of adrenalineoxidase, produced by imidazoline derivatives with different specificity of effect: Privine, Otrivine, 2020/n—sympathicomimetic; Priscol, 7337—sympatbicolytic; Antistine—antihistaminic. Abs.—concentration of drug; ord.—inhibition in per cent of control after 2 hours.

In general, the results of these experiments give no significant possibilities for the explanation of the specific differences, on the basis of general physicochemical behavior of this group of substances. The expansion of these investigations may change this situation. Also, in the present situation, they may give, just by their negative results, some indication on the behavior of the reactive system.

In the series of the aromatic imidazoline derivatives, substances are found with a chemically similar basic structure, and which possess very distinct specific sympathicolytic, sympathicomimetic, and histaminolytic effects. The specific effects of these substances are differentiated from others belonging to the same pharmacological group, by giving preference to definite points of attack of the specific system. Also, reverse effects are sometimes produced, and effects which belong to other systems can appear.

Consequently, the specific effect shows a system-restricted and an organ-restricted differentiation. This applies to the direct effect as well as to those which only appear as antagonistic effects in the presence of a change in the physiological function, produced by primary effective substances.

The establishment of the reaction equilibrium shown by the dosage-effect curves and time-effect curves and the action on correlated enzymes give no indication (or, only exceptionally, a parallelism) for the explanation of the specific effect.

The exclusion of especially the first-mentioned possibilities gives the basis for the conclusion that a specific reaction of the reactive cell system, independent of those mentioned factors, must be responsible for the specific effect of the substance. The reactive system, independent of what it is, must thus have a high degree of specific differentiated sensitivity towards certain substances of a very similar basic chemical structure and a whole series of others which have more or less different chemical structures. The fundamental difference between the types of specific chemical substances belonging to the same pharmacological group consists in the fact that nearly every group of effective substances has a predilection for only a series of the specific receptors. Thus, the reactive system must be able not only to create the specific effect but also the differentiation of those effects.

This supports the idea that the specific reaction on the cell is caused not so much by the fact that several sharply limited sites of reaction—*e.g.*, sympathicomimetic, parasympathicomimetic, and so on—exist, but that there is in every organ a complex of reactive places which can react with a whole series of chemical compounds in a primary or antagonistic fashion. Only if a substance can react on a definite combination of this reactive complex in a given organ does the specific effect occur. Substances such as adrenaline, histamine, acetylcholine, and others are preferred only because they may react in any organ in the specific way.

This view seems to be in accordance with that of one of the most prominent experts of this field, A. J. Clark,[15] who writes, "It appears to the writer, that the structure of the receptors on which drugs act is probably a subject of similar complexity but that the complexity has been masked by the fact that special attention has been paid to the action of acetylcholine and adrenaline, which are, however, widespread through the animal kingdom. In these peculiar cases a wide variety of tissues happen to possess receptors of similar patterns with which these drugs can react, and since the pattern is standardized, a fair regularity is observed as regards the action of antagonists; a systematic investigation of other drugs would probably reveal in most cases more erratic actions and antagonism." This, I assume, could be shown by the pharmacological problems discussed.

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THE MODE OF ACTION OF ANTIHISTAMINIC AGENTS IN THE SKIN

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Although the skin is the most accessible of our organs, we have considerably less precise physiologic data concerning it than we have concerning such deeply buried organs as the heart or a single neurone of the central nervous system. This is especially true of our knowledge of the function of histamine in the skin.

When the antihistaminic drugs became available, it seemed possible that a study of their action in the skin might offer a means of gaining further information regarding the role of histamine in the skin. This thought, plus the early demonstration of the efficacy of these drugs in certain disease conditions of the skin, led us first to make a study of the antihistaminic action of diphenhydramine hydrochloride (Benadryl) in the skin and then to extend the findings to other compounds of the antihistaminic series. Before these results are presented, however, the knowledge which has accumulated concerning the presence and action of histamine in human skin should be briefly considered.

1. The Presence of Histamine in the Skin

Extracts of human skin have been prepared by Harris,[1] by Pellerat and Murat,[2] and by Nilzén,[3] and after various degrees of purification the histamine content of the extracts has been determined biologically. The amounts of histamine found in the skin by these investigators are closely similar. Harris estimated the amount in normal skin to be about 10 micrograms per gram, with a range of 4 to 24 micrograms per gram for the different layers of the skin. On the basis of determinations in twenty-three subjects, Pellerat and Murat suggested a value for normal skin of about 20 micrograms per gram, with a range of 16 to 24 micrograms per gram. Nilzén tested samples of skin from thirty-three healthy subjects and found values ranging from 5 to 24 micrograms per gram, with a mean of 10.5 ± 1.0 $\sigma = 5.5$.

The active ingredient in the extracts prepared by these investigators was found by them to be pharmacologically and physiologically indistinguishable from histamine. Histamine, however, has not been isolated in pure crystalline form from skin. Until this is done and the identity of the crystalline product is established chemically, the conclusion that histamine is a normal constituent of skin must remain tentative. Still, it should be mentioned that no evidence has been presented to date which indicates that the H substance in extracts of skin is anything but histamine. The data so far accumulated, therefore, indicate that histamine is a constituent of normal skin and that it is present in skin in physiologically significant quantities.

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2. The Actions of Histamine in the Skin

If histamine is present in the skin, what is its function there? One approach to the problem that this question poses is to study the action of histamine in the skin.

A. The Triple Response. While several workers, notably Eppinger[4] and Sollmann and Pilcher,[5] had reported the effects produced by the introduction of histamine into the skin, Lewis and Grant[6] were the first to study the reaction in detail. They presented what is now the classic description of the action of this compound in human skin, "the triple response." The reaction consists of (1) a localized red spot at the site of injection of histamine; (2) a "flare," or diffuse erythema surrounding the site of injection; and (3) a "wheal" occurring in the region of the injection.

Lewis and Grant demonstrated that the localized red spot is due to intense vasodilatation in the small blood-vessels in contact with the histamine, that the flare is dependent on an axon reflex, and that the wheal arises as a result of increased capillary permeability at the site of injection of histamine. They showed that the response is similar to that given by a whole series of injurious stimuli. Their studies led them to conclude that such stimuli lead to the liberation in the skin of a substance which, in their opinion, is either histamine or a compound very similar to it, and that through it the standard stereotyped triple response is produced. Their work indicates one of the functions which histamine may perform in the skin—that of the common initiator of a defense response to a whole series of noxious stimuli.

B. Pain. In the investigations of Lewis and his associates on the skin, injury to the skin, antidromic impulses, pain, itching, and H substance were often closely associated. They, however, never ascribed to histamine or a histamine-like substance the role of chemical mediator of pain stimuli at pain nerve-endings. In fact, Lewis was apparently opposed to the view that histamine may act as an excitant of pain nerve-endings.[7] In so-called "susceptible" skin, some evidence was collected which suggested that a substance other than histamine was involved in the painful and hyperemic state of the skin.[8] Their results, however, led them to conclude that the triple response and the itching occurring after injury were both due to the release of a substance equivalent to histamine.[6,9] Lewis and Marvin[10] also found that antidromic impulses passing down sensory nerves produce vasodilatation by the release of a vasodilator substance. Thus, while Lewis and his associates did not regard histamine or a histamine-like substance as an excitant to pain nerve-endings, the association between H substance and pain-producing structures and phenomena in the skin was often intimate. Also, there seems nothing in their work which actually eliminates the possibility that histamine may be a chemical initiator of pain impulses at pain nerve-endings in the skin.

Rosenthal and Minard[11] have performed some interesting experiments which indicate that a histamine-like substance is liberated, not only when skin is injured, but also when it is stimulated by painful but not injurious stimuli. This substance was liberated from skin shavings of human beings and animals and from the cornea of rabbits when threshold stimuli were applied. These authors have also demonstrated that very small quantities of histamine produce pain when they are applied to denuded areas of the skin, when they are injected intradermally, or when they are applied topically to the cornea. Lambert and Rosenthal[12,13] have shown that a histamine-like substance may also be liberated from the appropriate cutaneous area when the cervical sympathetic chain or the splanchnic nerves are stimulated.

Ungar and his associates have carried on a series of interesting experiments using gastric secretion as an indicator of the liberation of histamine or a histamine-like substance during various types of stimulation of nerves and skin in dogs. Antidromic stimulation of sensory nerves was found to lead to the production of gastric juice after a latent period of about ten minutes.[14] It was not found possible, however, to identify definitely the substance liberated as histamine.[15,16] While electrical stimulation of the skin and exposure of the skin to cold were without effect, the action of strong irritants and plunging a paw into water at 50° C. for two minutes induced gastric secretion.[17] Such responses were not obtained after degeneration of the nerves of the paw.

In summary, the evidence accumulated to date indicates that histamine or a substance very like it (1) is present in skin, (2) participates in or initiates the triple response, which may be regarded as the response of defense by the skin to noxious stimuli, and finally (3) may be the chemical mediator or initiator of pain impulses at pain nerve-endings in the skin.

3. The Effects of Antihistaminic Drugs in the Skin

A. Effect on the Triple Response. The first of the synthetic antihistaminic substances, the Fourneau compounds 929, and 1571F, when studied by Staub,[18] were found to be without effect on the triple response to histamine. This finding was later verified by Burchell and Varco.[19] Soon after the introduction of the second group of antihistaminic compounds, the Rhone-Poulenc (RP) series, Parrot[20] showed that the response of the skin to scratching with a needle through drops of 1:100,000 solution of histamine was diminished by the oral administration of 0.2 to 0.4 gm. of Antergan (2339 RP). Vallery-Radot, Mauric, and Halpern[21] have shown in a study of six subjects that Antergan and Neoantergan are both capable of diminishing and in some instances of abolishing the response to histamine in the skin. They gave 0.5 gm. of each of the drugs, 0.4 gm. being administered by mouth and 0.1 gm. being injected intramuscularly.

Elias and McGavack[22] have tested the reaction to histamine in the skin of normal subjects before, during, and after the oral administration of 150 to 300 mg. of Benadryl per day. Histamine was introduced into the skin by means of a pin-prick through a drop of 1 per cent aqueous solution of histamine. Doses of 150 mg. of Benadryl per day definitely reduced both the wheal and flare reactions to histamine. In some cases, after ten days of treatment with 300 mg. of Benadryl per day, the wheal and flare reactions were completely abolished. Using a similar technic, Harley[23] found that after the oral administration of 300 mg. of Benadryl on one day, followed by 150 mg. a day for six days thereafter, the reactions of the skin to histamine were reduced to a fourth at the end of the first day and to about three-fourths of their original size by the sixth day.

A month or two after Elias and McGavack had reported their results, Friedlaender and Feinberg[24] published observations that they had made on the effect of Benadryl on cutaneous reactions in man. Histamine wheals were induced as a routine by the scratch technic. They found that 100 mg. of Benadryl by mouth did not have a consistent effect on the size of wheals and had no effect on the size of flares induced in response to scratches made through histamine solution. Pretreatment of the area by exposing the scratch site to a 50 per cent solution of the antihistaminic agent for ten minutes did, however, produce a pronounced reduction of the size of both the flare and the wheal. Similar results were obtained with flares and wheals produced in sensitive patients by ragweed antigen. Preliminary studies with tripelennamine hydrochloride (Pyribenzamine) indicated that it was at least as effective as Benadryl in reducing the whealing reactions. Arbesman, Koepf, and Miller[25] found that the oral administration of Pyribenzamine in doses of 50 to 150 mg. decreased the size of histamine skin wheals in eighteen of twenty-eight subjects.

Cohen and associates[26] administered histamine to the skin in various concentrations by iontophoresis. When 50 mg. of Benadryl were given orally four times a day for one to seven days, solutions of histamine which were two to thirty-two times as strong as those used in control tests were required to produce a whealing reaction in the skin. Benadryl given by ionotophoresis also raised the cutaneous threshold to histamine. Aaron and Abramson[27] observed that areas of skin treated with 5 and 10 per cent solutions of Pyribenzamine by iontophoresis lost or nearly lost the whealing response produced in normal skin by the iontophoretic administration of 1:10,000 and 1:100,000 dilutions of histamine phosphate. While the first two synthetic antihistaminic compounds studied, 929 F and 1571 F, are apparently without action on the triple response, the investigations summarized in previous paragraphs clearly show that the more recent members of this group of compounds, Antergan, Neoantergan, Benadryl, and Pyribenzamine, do exert an effect on the action of histamine in the skin. They all reduce the reactivity of the skin to histamine. With the exception of the work of Cohen and associates, the results allowing this conclusion, while they have demonstrated the points of interest to the authors, have, for the most part, been qualitative and have, in the main, been based on observations of the whealing response to histamine. There are few or no definitive data in the literature dealing with the effect of the antihistaminic compounds on the flare component of the response to histamine.

B. Action on Pain Nerves. Rosenthal and Minard[11] were the first to demonstrate the anesthetic action of antihistaminic drugs. They noted that thymoxyethyldiethylamine (929 F), when injected intracutaneously or applied locally to denuded skin in 1:200 dilution, produced local anesthesia similar to that obtained with 1:100 solution of procaine hydrochloride but of longer duration. When given orally or rectally in sufficient amounts to dogs, monkeys, and guinea pigs, the drug generally abolished the pain responses to pinching, pricking, and cutting and raised the electrical threshold of the skin, without, however, affecting that of somatic, sensory nerve trunks. Rosenthal, Minard, and Lambert[28] have confirmed these findings and have noted a similar reduction of the threshold of abdominal visceral sensation after administration of the drug.

Burchell and Varco[19] observed some general lowering of the pain threshold in dogs receiving large doses of thymoxyethyldiethylamine (929 F) subcutaneously. In human skin, anesthesia occurred over blebs produced by the intracutaneous injection of 929 F and 1571 F, while none occurred when the drugs were given subcutaneously.

Very few tests for anesthetic properties have been made with the newer antihistaminic compounds. The French pharmacologists apparently have not been much interested in this potentiality of the compounds. Halpern,[29] in his extensive report on Antergan, simply asserted that compounds of the series 2325 RP have significant local anesthetic activity, but he did not give data in support of the statement and made no further comment regarding it. Clinical investigators in France have suggested and observed on a number of occasions that the administration of antihistaminic compounds will produce a reduction of the pain of shingles and certain causalgias.[30,31] Dews and Graham,[32] in England, reported that Neoantergan, when given intracutaneously to guinea pigs, produced local anesthesia and that its local anesthetic potency was approximately three times as great as that of procaine. They also noted some mild though variable analgesia in rats receiving the drug.

4. Summary of Experimental Data

Because of the possible role of histamine at pain nerve-endings in the skin, it was decided to investigate further the anesthetic action of the antihistaminic compounds. Anesthetic activity of these compounds was first tested. Then experiments were done to determine whether or not there was a relationship between anesthetic action and antihistaminic effects.

Benadryl (diphenhydramine hydrochloride) was used in the first series of experiments. The results obtained with this compound were then extended in a second study, in which a comparison was made of the actions of Benadryl,* Pyribenzamine* (tripelennamine hyrochloride), Neoantergan* (ethylenediamine, N-p-methoxy-benzyl-N′, N′-dimethyl-N-2-pyridylmoleate), and the two compounds derived from thiodiphenylamine,* beta-dimethylaminoethyl-N-thiodiphenylamine (3015) and beta-dimethylamino-alpha-methyl-N-thiodiphenylamine (3277) of Rhone-Poulenc.

It was first observed that when Benadryl was injected into the skin local anesthesia was produced at the site of the injection. Benadryl, in dilutions of 1:500, 1:1,000, 1:5,000, 1:10,000, and 1:20,000, was found to possess anesthetic effects in the skin equal to procaine in dilutions of 1:200, 1:400, 1:800, 1:1,600, and 1:3,200, respectively.[33]

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* The drugs used in this study were kindly provided by the manufacturers: Benadryl by Parke, Davis & Company; Pyribenzamine by Ciba Pharmaceutical Products, Inc.; and Neoantergan and the thiodiphenylamine derivatives by Société Parisienne d'Expansion Chimique Spécial.

The effect of the antihistaminic drugs on the size of flares resulting from the intracutaneous injection of histamine was used as a means of quantitative assay of antihistaminic action. The flares were produced under standard conditions and the areas were measured from photographic records.

In the first study, when the action of Benadryl only was being tested, control flares were always made on the same occasion as the test flares. In the more complex series of studies, in which the five antihistaminic agents were compared, space did not permit simultaneous inclusion of the control flares and, for this reason, they were made on other occasions. An extensive series of observations was made to determine the variability in the size of control flares made simultaneously and at different intervals of time to cover the experimental circumstances. The results obtained led to the development of standard assay procedures applicable to the experimental conditions.

The reduction in the size of histamine flares following the oral, intravenous, and intradermal administration of Benadryl was first confirmed. Experiments designed to determine the degree of dependence of the flare-reducing effect of Benadryl on its anesthetic action in the skin were then carried out. By the use of the assay procedures developed during the study, the flare-reducing properties of a standard local anesthetic agent (procaine hydrochloride) were tested. The observation first made by Lewis and Grant,[6] that cutaneous anesthesia induced by procaine reduces or eliminates histamine flares, was confirmed. The possibility arose that the antihistaminic action of Benadryl injected into the skin is dependent on the anesthesia it produces. If this were the case, then equianesthetic doses of Benadryl and procaine should have similar flare-reducing effects. When tests were made, however, it was found that Benadryl consistently produced a more pronounced reduction in the size of the flares than procaine of equianesthetic strength. Some separation between the anesthetizing action and the flare-reducing action of the drug was indicated.

By the use of similar doses of procaine and Benadryl, tests were then made of their effects on size of flare after the anesthesia that they produced in the skin had subsided. It was found that even when the anesthetic action of Benadryl in the skin had disappeared, its flare-reducing effects persisted and indeed the flare-reducing action of the drug was as potent in the absence as in the presence of the anesthesia. On the other hand, the flare-reducing action of procaine, after its anesthetic effects had disappeared, was slight or absent. The effect of Benadryl on histamine flares was not then directly dependent on the local anesthesia that it produced in the skin.

In the second study, the flare-reducing potencies of the five drugs (Benadryl, Pyribenzamine, Neoantergan, 3015, and 3277) were first compared. When similar molar concentrations of the drugs were used, it was found that Pyribenzamine and Neoantergan had the greatest effect on the size of the flares, while Benadryl was intermediate between these two drugs and the two thiodiphenylamine derivatives, 3015 and 3277, which had the least and approximately equal effects on size of flare.

The anesthetic actions of the drugs were then compared. It was found that they all produced local anesthesia in the skin when injected intradermally. Similar molar concentrations of the drugs were used, and the duration of anesthetic effect which they produced was tested. Based on this measure of anesthetic potency, 3277 and 3015 had nearly equal and, at the same time, the most potent action of the series of drugs. There were only minor differences between Neoantergan, Benadryl, and Pyribenzamine, but they were all distinctly less effective than the thiodiphenylamine derivatives. Since the order of potency was the exact reverse of that noted for the flare-reducing action of the drugs, the conclusion was drawn that the effect of the drugs on size of flare is not directly dependent on their anesthetizing action in the skin.

As a final step in the study, the flare-reducing actions of the compounds were tested one hour after their injection into the skin, when the anesthesia that they had produced had subsided. Even in the absence of anesthesia, the drugs continued to show considerable flare-reducing activity.

The experiments allow the conclusion that the flare-reducing action of the antihistaminic agents studied is not directly dependent on the presence of the local anesthesia which they produce in the skin. Lewis and coworkers have shown that the flare produced by the injection of histamine is due to an axon reflex. It seems most probable that the flare-reducing action of the antihistaminic compounds is due to some effect on nervous tissue in the skin. A direct action on the blood-vessels might be involved; but this seems unlikely, for the antihistaminic drugs could hardly have diffused a sufficient distance from the site of their injection to reach all the minute blood-vessels ordinarily involved in a histamine flare. Also, if a direct effect on blood-vessels were the cause, one would expect to see, at times, no flare in the immediate vicinity of the site of injection of the antihistaminic drug and flaring at some distance from the injection. This was never observed. Some action on nerve tissue, therefore, seems the most likely explanation of the drug effects.

The separation between antihistaminic and anesthetic actions may then be a result of differences in degree of action on nerve tissue rather than a separate action on different tissues. Following this argument, it may be concluded that the impulses which are normally generated by the injection of histamine and are carried antidromically to the blood-vessels in the flare region are apparently interrupted or are not initiated when an antihistaminic compound is present. This dislocation persists even after sensitivity to pain, as tested by electrical stimulation, has returned to the skin. The mechanism of initiation of the impulses giving rise to the flare may be closer to the minimal disturbance which will excite the nerve channels involved than the disturbance induced by electrical stimulation of the skin. Under such circumstances, concentrations of the antihistaminic compounds which produce no effect on pain-impulses might, nevertheless, block flare-producing impulses. Such an effect offers an explanation of the antiflare action of the drugs following their oral and intravenous administration, when no anesthesia in the skin is present. It also serves to explain the persistence of antiflare action after a subsidence of anesthesia following the intracutaneous injection of the drugs. Further experimentation will be required, however, to determine whether this is the mechanism of action of these drugs and whether their action on nerve-tissue is directed toward nerve-endings or toward nerve-trunks in the skin. The elucidation of such points as these will undoubtedly lead to a clearer understanding of some of the effects of histamine in the skin.

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ANTIHISTAMINE THERAPY. EXPERIMENTAL AND CLINICAL CORRELATION

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In a discussion or evaluation of an antihistaminic drug from the clinical standpoint, we should ask ourselves three major questions: (1) What manifestations are helped, to what extent, and what are the limitations of its use? (2) What are the nature, extent, and significance of the undesirable actions of the drug, and how can they be remedied? (3) What are methods by which such a drug can be evaluated prior to clinical experience with it? In other words, what experimental methods will give us the best correlation with clinical results?

The purpose of this paper is to present the highlights of the facts pertaining to each of these questions. The clinical and experimental findings which I shall discuss are primarily those encompassed in my own experience in my practice and laboratory. For the most part, the points elaborated here will concern themselves with the antihistaminic drugs as a group, rather than with the individual drugs. Because experience with many of these drugs is in its initial stages, I shall refrain from making any sharp clinical or experimental comparisons, which could be easily modified by future more extensive observations.

I. Clinical Results

Seasonal Hay Fever. With most drugs, about 60 to 80 per cent of the seasonal hay-fever patients obtain some degree of improvement in their symptoms.[1,2,3] The degree of relief varies from slight to excellent, but rarely is it complete. It is exceedingly important to realize that the incidence and degree of relief are determined by many variables besides the potency of the drug. One of the most variable is the individual patient. Irrespective of the severity of the symptoms, some patients do not respond favorably to the antihistaminic drugs. What is even more difficult to understand is that this individuality may extend to a selective response to some members of the antihistamine group while, to others, the response is unfavorable. In another person, this selective response may be reversed. It is this peculiar behavior, together with individual differences in the production of undesirable symptoms, which makes it of advantage to possess a range of antihistaminic drugs from which to select the most desirable one for the particular patient. As a corollary, it is also this characteristic which should encourage the production of a reasonable number of new antihistaminic agents, even though their incidence of effectiveness may be no greater than, or as great as, previous products.

In evaluating any antihistaminic drug in hay fever, one must bear in mind that a number of conditions determine its action. Some of the hay-fever symptoms, such as sneezing and rhinorrhea, are readily helped, while others, such as nasal blocking and headache from edema of sinus mucosa, may be resistant. The type and variety of hay fever make considerable difference. Grass-pollen cases will respond more easily than ragweed cases, and mild ragweed cases will do better than severe ones. The severity of the particular season has an influence. For example, some ragweed-sensitive patients who responded well in the 1946 ragweed season in the Central States did not do so well in 1947, for no other reason than that the latter season was more intense.

Geographical differences in pollen prevalence have a profound bearing on the response of hay fever to antihistaminic drugs or to desensitization therapy. It is one thing to combat symptoms in a locality which has a pollen count of 50 at the height of the season and another to meet conditions in a locality like Chicago, where the count at the height of the season is 800 or more and a count of 50 generally occurs before August 15, the date which the average layman has accepted as the official opening date for the season.

It is also a common experience that the antihistaminic drugs which have been so helpful at the beginning and height of the hay-fever season may be totally ineffective during the later stages, when the swelling of the mucosa is apparently less labile and when weather factors play a predominant role. From all these facts, it is easy to see why patients who have had their hay fever mitigated by desensitization therapy respond much better to antihistaminic drugs than do the undesensitized patients.

Other conditions having to do with physiological states determine the efficacy of these drugs. A dose of the drug taken on an empty stomach may be markedly effective, whereas the same dose taken after meals may fail to work. There are other less definable body states which apparently have an influence in causing variation in effectiveness of these drugs from time to time in the individual patient.

Perennial Vasomotor Rhinitis. The incidence of relief in chronic vasomotor rhinitis is from about 30 to 60 per cent with most drugs, depending on the drug and the nature of the cases encountered. The question arises: Why is the incidence of relief less than in the seasonal cases? One or two possible explanations are offered. Most patients with perennial rhinitis present the symptoms of persistent nasal blocking as the major complaint. This complaint resembles the condition that prevails in some patients at the end of the hay-fever season, where we have also observed that the effectiveness of these drugs is diminished. To substantiate this view further, we have noted that very early cases of perennial vasomotor rhinitis, whose major symptom is sneezing, respond as well as the seasonal cases. Another possible explanation is that in perennial rhinitis we may be dealing with an appreciable percentage of instances of intrinsic causes which may perhaps not respond in the same manner as extrinsic cases.

Asthma. The typical asthma attack is influenced very little by the oral use of the present antihistaminic drugs. This fact is so generally overlooked and so commonly misunderstood that one cannot overemphasize it. In the past few years, I have had not a week go by without observing one or several new patients who had been having persistent asthma without relief and whose major medication had been Benadryl or Pyribenzamine. Frequently, a few doses of the older remedies—ephedrine, aminophylline, epinephrine, or iodides—were sufficient to break up the attack. On the other hand, the addition of an antihistaminic drug to the other therapy may make for more effective relief in selected instances of asthma. The spasmodic cough without dyspnea or wheezing, which is a type of bronchial allergy frequently seen in children, responds favorably to these drugs in 25 to 60 per cent of the cases.

Two likely explanations suggest themselves for the lack of response of asthma to these drugs. It may be that in asthma we are dealing with a mechanism different from, or additional to, histamine. Another possibility is that the local release of histamine in the bronchi is of a magnitude greater than can be combated by the amount of antihistaminic drug which can be tolerated. It is possible that new drugs with much less toxicity or greater potency may permit the solution of this problem. It is also possible that the topical application of the antihistaminic drug by aerosols might solve this situation. We have experimented clinically with aerosols and have had some encouraging but inconstant results. The obstacle may lie in the difficulty of getting a sufficient amount of the drug to the bronchi by the ordinary simple methods. We have also experimented with micronized drug inhalations, which offer some interesting possibilities. Meanwhile, we have turned to extensive experimentation, in guinea pigs, with aerosols of the drugs in their ability to prevent bronchospasm induced by histamine aerosols. We have studied the concentrations required and the effectiveness, tolerance, and duration of action of a large series of antihistaminic drugs. The detailed findings will be presented elsewhere.[4] Here I wish merely to call attention to the following facts: aerosols of these drugs are effective in the prevention of bronchospasm from histamine aerosols; the duration of action is short, averaging about an hour; and some drugs are very irritating when administered in this manner.

Urticarial Dermatoses. Urticaria and angioneurotic edema respond well to these drugs, producing improvement in 60 to 80 per cent of the cases.[2,5,6] The itching is the symptom most consistently helped, while the edema may be improved moderately or not at all. In severe and dangerous angioneurotic edema, such as edema of the larynx, more prompt results can be expected from epinephrine hypodermically. It is best to give the latter and the antihistamine drug at the same time. The delayed serum-sickness type of reaction frequently encountered a number of days after the administration of serum, penicillin, or sulfonamides also responds well to these drugs. The joint symptoms, however, are more resistant. In all such conditions, the benefit is only temporary, lasting from three to eight hours with each dose. The syndrome pursues its usual course and duration. Dermographia is also benefited by such drugs, and the moderate dermographia, so frequently an obstacle to the interpretation of diagnostic skin tests in allergy, usually can be controlled by the prior administration of a dose or two of the drug.[6]

Allergic Dermatitis and Other Dermatoses. The itching of atopic dermatitis (flexural eczema) is helped in 50 to 80 per cent of the cases.[2] In some instances, the condition of the dermatitis may even improve, because of the discontinuance of scratching and the correction of excessive histamine damage. Some patients require medication only at night, while others gain more by the use of the drug several times daily. In many instances, the use of an ointment of the drug is of additional advantage.[7] Contact dermatitis responds also, but not as frequently, to the use of these measures. Pruritus ani and vulvae are frequently ameliorated by these drugs, and Pyribenzamine ointment has been found to be an excellent palliative remedy in pruritus ani. Many instances of other types of pruritus, as well as insect bites and dermatophytosis, also respond to the drug orally.

Miscellaneous Manifestations. Migraine headaches are only occasionally relieved. Gastrointestinal allergy is improved in some instances, although this action is often handicapped by the tendency of these drugs to produce gastrointestinal upsets in some patients. These drugs are helpful in the prevention of systemic reactions from antigen administration, but their help should be regarded only as moderate, since they do not prevent such reactions from large increments of the antigen. In the treatment of systemic reactions, epinephrine, ephedrine, and aminophylline are much more trustworthy, but the antihistaminic drugs may be of additional aid. In histamine reactions from cold, heat, and alcohol these drugs have been found of help. The erythema, itching, and some of the edema of dermatomyositis have improved with the use of these agents.

Other Factors in Treatment. As in hay fever, the relief obtained with the antihistamine drugs in the conditions listed is seldom complete and depends on many variants, some known and some unknown. The usual duration of action from a single oral dose is three to eight hours, although in some instances it may be longer. The different drugs vary some in this respect, and it is suspected that one or two in particular may produce unusually long relief. However, this will require more extended and concise studies. I am satisfied that the vast majority of reports of duration of action given by patients cannot be trusted. Many patients will report that a dose of the drug has relieved their hay fever for twenty-four hours. Actually, the condition is as follows: The patient has hay fever in the morning. Without the use of any medication it might last only a half-hour to two hours, not to recur until the following morning. If the antihistaminic drug is administered and the symptoms disappear in about an hour, not only is it impossible to determine the duration of action, but it is even dubious whether the drug has produced any relief. The dose administered, the potency of the drug, the individual responsiveness of the patient to the drug, and the severity of the symptoms are factors which influence the duration of action.

Increasing experience with these drugs indicates that their continued use tends to diminish the effectiveness of therapeutic response in many instances. Whether this is due to a true tolerance or to psychic factors is not certain. It is possible that, once the novelty of a partial relief has worn off, the patient centers his attention on the unrelieved portion of his symptoms.

Dosage and Administration. Of the drugs studied by us thus far the effective average adult dose of most of them has been 50 mg. With some drugs, it has been possible to obtain results at times with 12.5 to 25 mg., while, with other drugs, 100 mg. doses were usually required. A number of additional factors determine the dose. Among these are the severity of the symptoms, whether the medication is taken on a full or empty stomach, and the limitations due to its toxic effects. There are, no doubt, additional unknown factors which determine the effective dose. Children tolerate proportionately high doses. Children over 10 usually tolerate adult doses, and sometimes better than adults. Younger children usually respond to 50 per cent of the adult dose, while infants as a rule require 20 to 40 per cent of the adult dose. In most instances, the drug should be administered as needed, rather than given at stated regular intervals. This will obviate unnecessary medication and will reflect more accurately the effectiveness of the drug. There is no excuse for administering the drug several times daily for an allergic attack which recurs only periodically.

Some physical and chemical considerations are of practical importance. The pill is the common form of such medication; yet, because of the bitter taste of most of these drugs, the pill becomes objectionable, unless it is sugar-coated. Some of the pills are so soft and unpleasant that the patient prefers other drugs which may be less potent but which come protected in capsule or by a pleasant coating. Liquids, usually elixirs, are also handy forms of medication. The amount of drug in one unit (capsule or tablet) has a definite bearing on the success of the therapy. If, for example, the usual effective dose of a drug is 50 mg. and it is dispensed in 25 mg. capsules or pills, a certain number of the patients will fail to take the prescribed 50 mg. because they will suspect it to be a double dose, even though they have been advised to the contrary previously. Thus, such patients may contribute to a reduction in the figures in the evaluation of the drug.

Hypodermic injections have been used, particularly of Benadryl, Antistine, and Pyribenzamine. They are seldom indicated but may be of use in emergencies. They should be employed with great caution, since their toxic action is not yet fully appreciated or understood. Aerosols may be of help in some cases of asthma. Topical applications in the nose and eyes may ultimately be of some aid, but in most instances and with most drugs the primary irritation is very objectionable. Applications on the skin in the form of ointments have been found valuable by us.[7] This came as an outgrowth of our experience with the inhibition of the dermographic wheal, when a drop of a solution of an antihistaminic drug was applied previously to the intact skin. It is possible that other forms of application of these drugs may also be of advantage.

II. Side Actions

The usefulness of the antihistaminic drugs is considerably hampered by a number of undesirable effects.[1,3] These untoward actions, although differing in degree in different drugs, are, for the most part, similar to all and present a characteristic pattern.

Sedation. The most consistent side action of all antihistamine drugs is sedation, or sleepiness. Indeed, it is so common an attribute and so constantly present, if the size of the dose is large enough, that it may be considered as much a pharmacologic action of these drugs as are their antihistaminic properties. Some patients have discovered that they can substitute these drugs for barbiturates when they desire more complete hypnotic effects. This sedative action appears to be peculiar to man, and it is interesting to speculate on its possible significance. Among the possible explanations for this phenomenon, the following have been considered: (1) that histamine is normally the mediator of sensory functions and that displacement of histamine results in sedation and analgesia; and (2) that this has nothing to do with antihistamine functions. The degree of sedation may vary from a slight diminution in alertness to deep stupor. The drowsiness, grogginess, and diminished alertness may not only be disturbing to mental concentration but may diminish actual physical energy. Furthermore, the mental fogginess can result in serious hazards and may lead to accidents.[8,9,10] The intensity of the sedation is a difficult thing to assay, particularly in the milder forms, because of the individual interpretations. Although, after considerable trial of a drug, we can obtain an idea of its sedative index, an enormous amount of clinical material and time are needed for this evaluation. An objective method of determining the effect of a drug on sedation or alertness would be more satisfactory, and we are now planning such a procedure.

Other symptoms occur which may be variations of the sedative action. Lassitude, diminished alertness, and diminished powers of concentration are undoubtedly part of the same effect. These symptoms are not to be ignored, since they may have a profound effect on the quality and quantity of physical and mental output. Disturbed muscular coordination and aching of muscles, particularly of the eyes, are probably related symptoms. On several occasions, I have observed temporary sexual impotence produced by antihistamine drugs. It may even be that the irritability, nervousness, and palpitation are, in some instances, due to the attempt to overcome the sedative action.

Factors in Sedation. A number of factors contrive to determine the occurrence and degree of sedation. The individual drugs differ. For example, Benadryl produces sedation in 50 per cent, Pyribenzamine in 25 per cent, and such drugs as Antistine and NH-188 are remarkably free from sedation. Some persons are more prone to this effect than others. The sedative action is more pronounced in adults than in children. It is remarkable how often it happens that, when a mother and her youngster of 10 or 12 take the same dose of the drug, sleepiness results for the mother and not for the child.

The individual response to different drugs varies. One drug will cause sedation in A and not in B, while another drug may produce the reverse effects. A number of other conditions determine sedative action. Taking the drug after meals may produce no toxic symptoms, while the same dose on an empty stomach may result in profound sedation. Conditions of weather, state of fatigue, degree of physical activity, and use of coffee or tea may affect the sedative action. It is common experience that, after the first few doses, a tolerance to the sedative effect may be acquired. No doubt there are other factors as yet unknown which have a bearing on this action.

Can the sedative action of an antihistaminic drug be appraised by acute toxicity experiments on animals? The obvious answer from the known pharmacologic data is "No." Clinical assay of sedation presents a number of difficulties. If the observer fails to question the patients, many instances of sedation are missed. If he does question them, the objection may be raised that the suggestive influence may be misleading. Many patients are very poor observers and fail to note anything unusual even if they stumble about in a fog. On the other hand, many individuals are very prone to misinterpret symptoms. Objective tests of the drugs on human alertness will have to be developed. Another possibility is the determination of sedative effects in monkeys or apes.

Correction of Sedation. Frequently, difficulties with sedative effects can be corrected by substitution of other antihistaminic drugs. Where this is not possible, the addition of stimulants may solve the problem. Racemic and dextro-amphetamine, desoxyphedrine, ephedrine, aminophylline, coffee, and Benzadrine inhalers constitute a choice of approaches. But this balancing of two opposing effects is far from a simple problem. No type or amount of stimulation fits all stages of sedation encountered. Furthermore, if the stimulant action is too great, a new train of undesirable symptoms ensues. Then there is the very delicate question of balancing the duration of action of the two opposing drugs, which seldom seem to neutralize each other completely for the entire period. It is not at all surprising, therefore, that we regard this problem as being far from settled. We believe the difficulty will not be solved until the molecular structure of the antihistaminic drug is properly modified to remove this undesirable activity, providing the sedative action is due to the drug itself.

Other Toxic Effects. Dizziness is a common symptom. Dryness of the mouth, throat, and nose is a frequent symptom and, if not too marked, may be of advantage, particularly in allergic rhinitis. Sometimes, however, it reaches undesirable proportions. Nervousness, excitability, insomnia, and palpitation are complaints of some persons, although oddly enough the same people will at times complain of sleepiness from the same drug. Disturbance of appetite is a common effect and gastric discomfort is not infrequent. At times, abdominal pain and diarrhea may result. Three or four instances of convulsive seizures have been described. It should be noted that, thus far, all drugs examined by us are local anesthetics. Although one may speculate on the possible relationship of the anesthetic and antihistaminic functions,[11] quantitative and qualitative experiments do not support the view that the one type of function is dependent upon the other.[12]

In some patients, we have observed marked burning in the epigastric and esophageal regions, particularly with one drug. Burning on urination has been noted occasionally. A few instances of dermatitis[13] have been encountered. More serious and delayed toxic effects, perhaps hematopoietic,[14] hepatic, or neurologic, may ensue as observations continue. Thus far, the reported serious effects are meager, but it is not amiss to emphasize that these are potent drugs whose pharmacology, toxicity, and mode of action are not entirely understood. A cautious observant attitude with any of the new drugs may save us from calamities.

III. Methods of Evaluation

An important step in the launching of a new drug is its assay or evaluation prior to release to the general medical profession. Let us examine the methods available for the antihistaminic drugs in order to see which may be of significance and which may have the greatest bearing on the clinical problem.

Laboratory Methods. Many laboratory methods have been proposed, but I shall briefly discuss only a few of them. The most common method concerns the protection conferred by the injection, usually intraperitoneally, of the drug in the guinea pig, to be followed by lethal doses of histamine intravenously. The first type of this technique aimed to find out the maximum number of lethal doses of histamine the animals will tolerate after the injection of a rather large amount of the drug. On that basis, a drug which protects against 50 times more histamine than another certainly does not possess such a relative clinical potency. We soon realized that such amounts of histamine are entirely too far removed from physiologic consideration in allergy and even in anaphylaxis. At the suggestion of Dr. Dragstedt, we modified this technique to observe the smallest amount of the drug which would protect the animal against 1 MLD_{100} of histamine, since that comes much closer to probable conditions of histamine liberation in anaphylaxis.

Another measure of antihistamine action concerns the prevention of bronchospasm from aerosolized histamine[15] after prior administration of the drug. The advantage here is that the animal does not have to receive a fatal dose of histamine and that the degree of histamine intoxication and symptoms more closely approach those of clinical allergy. We have further modified this technique by using aerosols of the antihistaminic drugs as protective agents.

The effect of the drug on the histamine contraction of the guinea-pig ileum is a favorite method of assay for antihistamine potency. In going over the literature and unpublished reports of various experimenters with this technique, one is struck by the fact that practically everyone has a different modification. One aims for total inhibition, another for 50 per cent, and still another for 75 per cent. The amount of histamine, the size of the strip, the timing, and many other factors vary. It is not surprising that one cannot safely compare the findings of one experimenter against those of another. Even when a standard technique is followed in one laboratory, such as ours, it is noted that innumerable and imponderable difficulties beset this procedure. It is a sensitive method, but, at best, it measures only the action of a drug on a piece of muscle, removed from living conditions and derived from an animal which is far removed from man. The inhibition of the depressor effect of histamine on the dog's or cat's blood pressure is another favorite method of study, but here again it is doubtful whether this is a measure of the drug's clinical worth.

Since antihistamine action is presumably not the only attribute of the drug desired, it is but natural that experimenters have turned to inhibition of anaphylaxis[16,17,18] as an indicator of the antiallergic properties of these drugs. This is probably as good a laboratory method as any. There are some objections to this technique, nevertheless, when one begins to make extensive comparative studies. The variability of anaphylaxis would mean that for conclusive work in all phases one would have to sacrifice tremendous numbers of animals. It has the further objection that in intensity of action it gets a considerable distance away from conditions which must prevail in connection with clinical allergy. The recent claim that antihistaminic drugs have an inhibiting effect on experimental lesions of serum sickness and periarteritis nodosa in the rabbit deserves further investigation.

One might expect that anaphylaxis *in vitro* would solve these problems. However, we have found such marked variability in sensitivity as to make many of the quantitative tests invalid. Since most of the allergic reactions concern themselves with vascular changes resembling whealing, it was thought that assays of these drugs on histamine wheals in rabbits would furnish a more accurate means of assay.[19] The whealing in rabbit skin is of a poor order and difficult to measure, however, and in my opinion it is not a good method.

Whealing in Man. Since increased capillary permeability in man is the most common characteristic of allergy, I have thought that studies of the effect of these drugs on histamine and other wheals on the human skin might give us a more accurate reflection of the activity of a particular drug. Long ago, we had shown that a solution of an antihistaminic drug applied to a scratch a few minutes prior to the application of a histamine solution inhibited or diminished the wheal from the latter.[20]

It is worthy of note that the whealing from other causes, such as antigens, codeine, atropine, eserine, *etc.*, were similarly inhibited. The procedure for assay by this method in its simplest forms is as follows: Several rows of scratches about one-quarter inch in length are made on the back of a subject. A drop of a standard solution of the drug to be tested is placed on each of six scratches in one row. On other rows, different antihistaminic drugs are placed for comparison. At the end of about 10 minutes the solutions are washed off. Each row of scratches is then treated with serial dilutions of histamine (usually from 1:1,000 to 1:32,000 histamine in terms of base). One row is treated with histamine without previous pretreatment with the antihistaminic drug. The height and degree of inhibition give us an idea of the activity of the drug.

We believe this method and modifications of it offer probably a closer approximation than any others of the anticipated behavior of the drug in allergy. We are utilizing this method not only in comparative evaluations of the antihistamine effects of various drugs, but also to study the quantitative relationship between histamine and antigen reaction in order to procure information which might throw light on the mechanism of the allergic reaction and on the mechanism of action of these drugs.[21] At this time, we shall not attempt to give details of our findings, except to say that by this means we have been able to demonstrate decisive and consistent differences between some of the antihistaminic drugs.

Clinical Assay. The final worth of a therapeutic product is, of course, judged by its behavior in a thorough clinical trial. I would like, however, to enumerate briefly some of the pitfalls and difficulties attending evaluation by clinical means. All the variables which we discussed previously as affecting the therapeutic results from the antihistaminic drugs in hay fever operate to modify the clinical assay of such drugs. These include the severity of the disease, its stage, the type of hay fever, the severity of the particular season, the geographical location of the trial, and whether the patients are those who have partial immunity from desensitization or those who have been entirely untreated. The size of the dose used reflects on the general results, and even the amount of drug in one tablet or capsule has influence on the total amount of drug the patient is apt to take. The reliability of the patient is of great importance, and frequently in suspicious cases we have been able, by one procedure or another, to decide that the patient's report is not to be trusted.

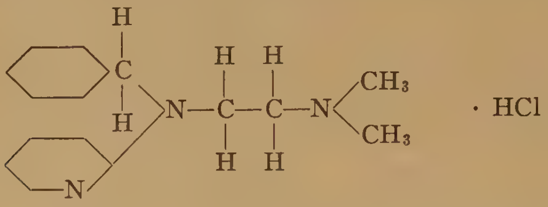
Still more difficult to judge is the duration of action of these drugs clinically. Only now and then can one obtain an allergic patient whose symptoms are so unvarying during the twenty-four-hour period that we can tell with certainty the duration of action of a single dose of the drug. I have already referred to the difficulties in judging the sedative action of these drugs. Only by a sufficient number of cases, over a fairly extended period, with a methodical approach, and in the hands of a critical and unbiased observer can these factors be evaluated.

In our method of clinical assay, several features were embodied: (1) The patients were usually instructed to take the drug as needed rather than at set intervals. (2) In almost every instance, the action of the drug thus tried was compared, at different times in the same patient, with one or more other antihistaminic and antiallergic drugs. (3) In most instances, during the period of evaluation the patient reported to the office about twice weekly, during which visits his symptomatology for the preceding several days was discussed and judged in terms of other variables, such as the pollen or mold count. (4) Placebos and substitute drugs were sometimes given when the patient's report was open to doubt. (5) The drug was tried under various conditions, in varying doses, and by various schedules in the same patient. (6) Our final grading of the patient's results was based on the following considerations: if a patient was improved once or twice, and not improved the majority of times under more strenuous conditions, he was graded as unimproved; if the relief occurred most of the time, but was absent during special periods, such as the height of the pollen season or in the congestive post-season phase, this was set down as an improvement. The results were graded as "slight," "moderate," or "excellent," the latter indicating that the symptoms were completely removed. Most results fall into the "moderate" classification, and rarely does a patient have complete freedom of symptoms.

IV. Individual Drugs; General Characteristics

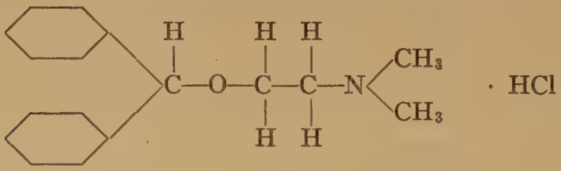
As a result of extensive experimental and clinical work with a large number of antihistaminic drugs, we have accumulated considerable data. Because of limited space and because some of the results are incomplete, I shall refrain from presenting a complete comparative report. Instead, I shall point out briefly in general terms the chief characteristics of each drug. This list of drugs, with the exception of those that were confidential at that time, consists of those with which I had experience up to 1947.

Pyribenzamine. (N′pyridyl-N′ benzyl-N-dimethylethylenediamine HCl) [17,18,22–25]



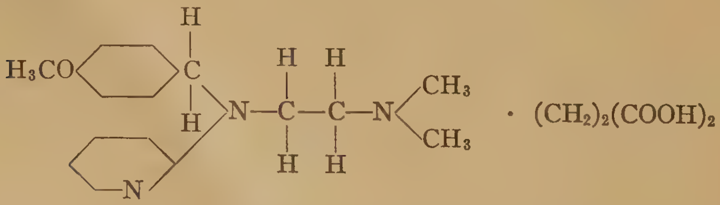
Shows potent antihistaminic action in animals, excellent action as an aerosol in guinea pigs in the prevention of histamine bronchospasm, good anti-anaphylactic activity, and excellent efficiency in inhibiting histamine whealing in man. It stands at the top of the list in clinical efficacy and reliability. Its side actions, particularly sedation, may be called average, in terms of the entire series.

Benadryl. (B-dimethylaminoethyl benzohydryl ether HCl)[2,3,5,15,16]



Antihistamine and aerosol action and antiwhealing effect good, but less striking than Pyribenzamine. Antianaphylactic action compares favorably. Clinical efficiency good, but not as effective as Pyribenzamine in nasal allergy. Sedative action is marked and frequently interferes with therapy.

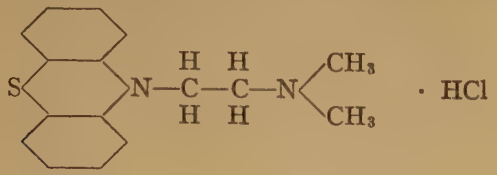
Neoantergan. (N-p-methoxybenzyl-N-dimethylaminoethyl α aminopyridine maleate)[3,26–28]



Antihistamine action is very high in terms of multiple lethal doses, but about the same as Pyribenzamine on basis of 1 MLD and aerosol method. Antiwhealing and antianaphylaxis are about the same as Pyribenzamine.

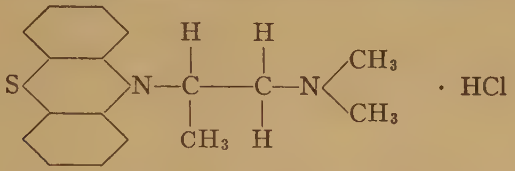
Clinical effectiveness good. Sedation moderate and gastrointestinal irritation not infrequent.

3015RP. (N-dimethylaminoethyl thiodiphenylamine HCl)[29,30]



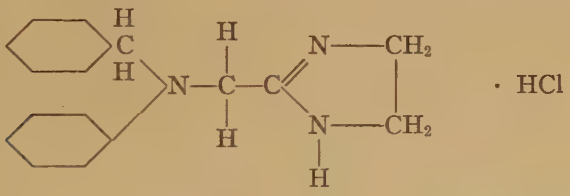
Good antihistaminic and antianaphylactic action. Poor antiwhealing effect in man. Negligible clinical response.

3277 RP. (N-dimethylaminopropyl thiodiphenylamine HCl)[29,30]



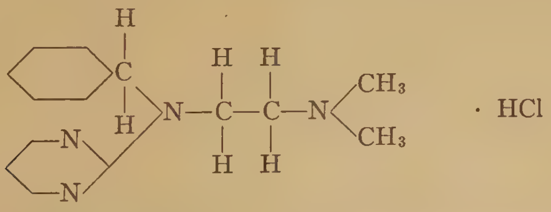
Antihistaminic effect on basis of 1 MLD_{100} comparable to Benadryl, Pyribenzamine, and Neoantergan. Aerosol is very irritating and has poor antiwhealing effect in man. Possible clinical effect completely overshadowed by extreme sedation even in small doses.

Antistine. (2-(N-phenyl-N-benzyl-aminoethyl)-imidazoline HCl)[1,31,32,33]



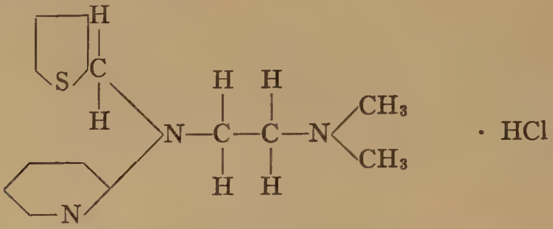
Antihistaminic and antianaphylactic action is much less than in the other drugs mentioned above, although more complete data with larger doses of the drug are needed. Antiwhealing action is moderate. Clinical effect is moderate and requiring large doses. Toxic action in man is very slight.

NH-188. (2-(N-dimethylaminoethyl-N-p-methoxybenzyl)-aminopyrimidine HCl)



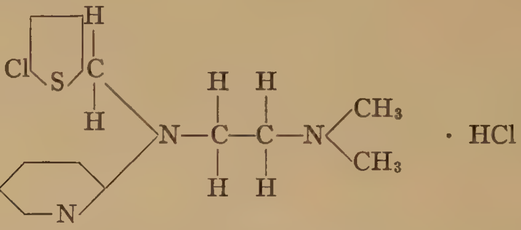
The antihistaminic, antianaphylactic, and aerosol action are very moderate. The antiwhealing property is moderate. Clinical effect is moderate, requiring larger doses than most drugs. An outstanding characteristic of this drug is its relative freedom from undesirable side actions.

Thenylene and Histadyl. (N-(α-pyridyl)-N-(α-thienyl)-N′, N-dimethylethylenediamine HCl)[1,34–36]



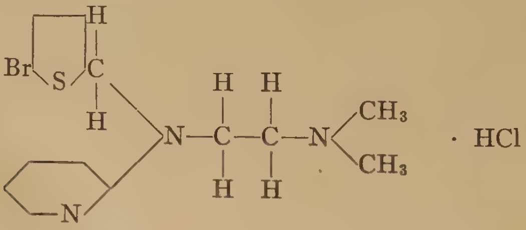
Excellent antihistaminic, aerosol, antiwhealing, and antianaphylactic effects. Good clinical effectiveness, but probably not quite so potent as Pyribenzamine. Sedative action about the same or slightly greater than Pyribenzamine.

Chlorothen. (N,N-dimethyl-N′-(2-pyridyl)N′-(5-chloro-2-thenyl) ethelenediamine HCl)[37,38]



Antihistamine action excellent. Antianaphylactic and antiwhealing effects good. Its clinical efficiency is very good, but perhaps not quite so high as Pyribenzamine. Its sedative action is about the same as or possibly slightly greater than Pyribenzamine.

Bromothen. (N,N-dimethyl-N′-(2-pyridyl)N′-(5-bromo-2-thenyl) ethylenediamine HCl)



The activities of this drug appear to be identical in all respects to those of Chlorothen.

Summary

(1) The antihistaminic drugs are valuable adjuncts in the symptomatic treatment of allergic manifestations. They should not and cannot replace

more basic and more durable immunologic therapy of elimination and desensitization, nor should other antiallergic drugs be discarded.

(2) The antihistaminic drugs possess undesirable side actions, of which the most troublesome is sedation. The possible remote toxic effects of many drugs are still not ascertained.

(3) Because of differences in individual clinical and toxic response, it is of advantage to have available a choice of a reasonable number of drugs. It is obvious, however, that there is a limit to the number of such drugs needed for this purpose.

(4) Opportunity still exists for the synthesis of the ideal antihistaminic drug, which will be sufficiently potent and entirely nontoxic.

(5) The clinical evaluation of these drugs presents a complex and difficult problem and requires, at the least, the painstaking and extended effort of unbiased and astute clinical observers.

(6) Many experimental methods of assay have been tried by the author and by others. Of all the assay methods tested thus far, we believe that the antiwhealing effect on the human skin will show the greatest correlation with clinical effectiveness.

(7) The advent of the field of antihistaminic drugs has opened up new questions concerning histamine function and new approaches to experimentation in many fields, including the mechanism of the allergic reaction.

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Discussion of the Paper

S. Walter Landau (*The Johns Hopkins Hospital, Baltimore, Maryland*): Our group at The Johns Hopkins Hospital has used fifteen different antihistaminic drugs clinically, and has tested most of these drugs in the intestinal strips of guinea pigs and against one lethal dose of histamine injected in the bloodstream of guinea pigs. Our clinical results can be summarized as follows:

In urticaria, relief ranged from good to excellent. Results were particularly good in penicillin reactions. In many cases of atopic and contact dermatitis and in pruritus ani, the drugs were very helpful in reducing or completely controlling the itching.

In hay fever and perennial allergic rhinitis, relief was from fair to good. The mild symptoms of patients who had had beneficial hyposensitization treatment were satisfactorily controlled. Patients who had poor results with hyposensitization treatment had only slight benefit from the antihistaminic drugs.

In bronchial asthma, relief was poor. This fact should be emphasized more than has been done so far. We have seen patients with asthmatic attacks suffer unnecessarily under prolonged treatment with such drugs, when administration of adrenalin or even one of the aminophyllin compounds would have relieved them at once. We had an unusually large number of patients who had asthma late in the ragweed season in 1947. Among them were many who had previously been relieved from their hay-fever symptoms by these drugs. This time, however, they failed to derive any relief of their asthmatic symptoms.

There was no striking difference in the clinical effect of the drugs. Some patients reported more benefit from one particular preparation and some from others. Dr. Feinberg has called attention to this individual responsiveness, and this factor deserves further investigation. One might think of interference with the action of the drug in the digestive tract under certain circumstances. We have noticed, also, that some drugs act more quickly than others. Locally, we have used Antistine in the eye and have had encouraging results in a number of cases.

Our animal experiments revealed that the drugs at our disposal can be grouped into three categories of potency. However, there was no parallelism with their clinical effectiveness. This is not surprising in view of the evidence, submitted in this monograph, pointing to factors other than histamine in allergy and to effects other than antihistaminic of these compounds.

We feel that the possibility of harmful effects of these drugs has not yet been completely ruled out. When Dr. Pasteur Valery-Radot visited our clinic in September 1947, he told us that four cases of agranulocytosis due to Neoantergan have been observed in France. Such reports, the unpleasant side effects of most of the compounds, and particularly their failure to relieve serious allergic conditions such as bronchial asthma present a challenge to further thorough studies.

GENERAL DISCUSSION*. I

By J. A. Wells

Northwestern University, Chicago, Illinois

Compounds which differ widely in their stated potencies as histamine antagonists frequently have been found to be of essentially equal potency in combating anaphylaxis and certain forms of allergic disease in which the action of histamine can be presumed to be of consequence. This apparent lack of agreement in potency has been used to support the notion that actions other than histamine antagonism are responsible for the effectiveness of these compounds in allergic disease.

Basic to such argumentation is complete confidence that the original estimations of potency of these drugs as histamine antagonists are reliable and valid. There is evidence that this is not the case. Stated values for the potency of these compounds are widely different and depend upon the circumstances under which the potency was determined. Each stated value must be qualified by saying that the potency is a certain figure if one employs a particular response of a particular tissue of a particular animal to a particular concentration of histamine in the presence of a particular concentration of the antagonist.

Thus, one of the fundamental requirements in the study of antihistaminic or antiallergic drugs is a reproducible and valid estimation of potency which is independent of the circumstances under which the estimate is obtained. Such an estimation of the potency of these compounds requires some knowledge of the mechanism of their action.

The histamine antagonists fail to meet the requirements of certain of the well-known types of antagonism, such as "physiological" or "chemical" antagonism, and we have thus assumed that their mechanism of action is what has been termed by A. J. Clark as "specific" antagonism, a more descriptive name for which is "competitive inhibition." The implications of such a mechanism of antagonism are that both the active drug and its antagonist compete for the same site of attachment in a tissue, the antagonist combining with this site without eliciting a response of the tissue. In more picturesque terms, the antagonist may be considered as acting the part of the "dog in the manger."

The antihistaminics fulfill one of the requirements of competitive inhibition by their ability to antagonize the action of histamine on certain tissues from which they are incapable of eliciting a response.

The competitive aspect of this type of antagonism implies that histamine, in sufficient concentration, will overcome the effect of a given concentration of the antagonist and that the antagonist, in sufficient concentration, will overcome the effect of a given concentration of histamine. To determine this, it is necessary to investigate the quantitative response of some tissue to histamine in the presence of a histamine antagonist. For this purpose, let us consider the contraction of isolated strips of guinea-pig ileum, subjected to the action of histamine in the presence of antagonists, such as Benadryl or Pyribenzamine.

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* The present discussion is based upon recent work carried out in collaboration with Doctors John S. Gray and A. Zadina.

With increasing concentrations of histamine, the responses of such strips increase greatly at first, then less and less, and finally approach a maximum. The response of this same strip to increasing concentrations of histamine can then be determined when various concentrations of the antagonist are present in the solution bathing the strip.

If one constructs free-hand curves showing the relationship between the concentration of histamine and the response at various concentrations of the antagonist, it is apparent that each curve passes through the origin and approaches the same maximum (FIGURE 1).

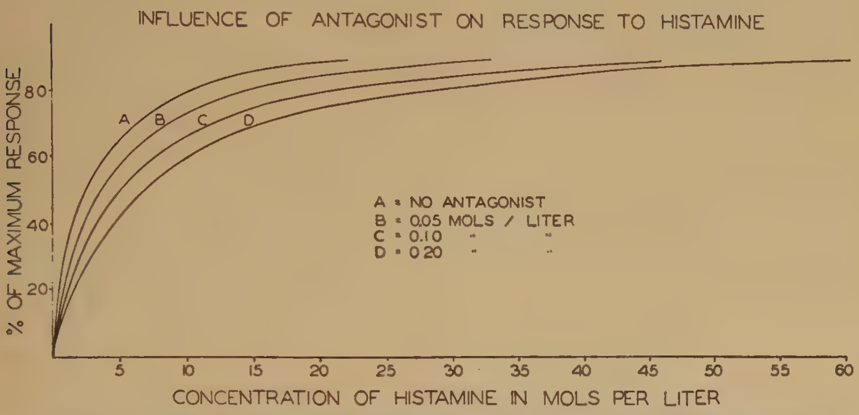

FIGURE 1. These curves are diagrammatic representations of the concentration of histamine required to cause various percentage responses of the isolated guinea-pig ileum in the presence of various concentrations of Benadryl. The various concentrations expressed as moles per liter on the graph are actually micromoles or moles $\times 10^{-6}$ per liter.

The fact that the maximum response which can be produced by histamine is unaltered by the presence of the antagonist implies that, regardless of the concentration of the antagonist, a sufficient concentration of histamine will overcome its effects. While each curve passes through the origin and has the same maximum, they differ in curvature and tend to flatten with increasing concentration of the antagonist. This implies that, regardless of the concentration of histamine, a sufficient concentration of antagonist will decrease the response which it produces. Thus, the histamine antagonists fulfill the requirements of competitive inhibition.

Competitive inhibition implies that some sort of easily reversible union occurs between the competing drugs and a receptor in the tissue for which both have an affinity. Such a union might be of the nature of a loose chemical bond or a process of adsorption. We should, thus, look to the laws governing chemical or adsorptive equilibria for the interpretation of a system of competitive antagonists.

In line with our principal objective, it is apparent that the correct estimation of potency of the antagonist must be in terms of the affinity of the antagonist for the receptor relative to that of histamine for the receptor.

If these affinities can be quantitated, then we have a quantitative estimation of the potency. The question is: Can this be accomplished?

The first step in answering this question is to determine the type of reaction between drug and receptor. A number of possibilities exist. The antagonist, as well as histamine, may combine in mono-monomolecular fashion with the receptor, in which case only two affinities (equilibrium constants) need be determined, one for histamine and one for the antagonist. This also applies if adsorption is representative of the type of combination. On the other hand, several molecules may be involved in the reaction, in which case several equilibrium constants will need to be determined.

Solution of the problem of the type of reaction which occurs comes from the application of the law of chemical equilibria to this system of competitive antagonists. By means of such an application, it has been possible to reject the notions that competition occurs on the basis of either competitive mono-monomolecular chemical reactions or competitive adsorption. In addition, some of the more complicated types of chemical reaction may be rejected as not applying to the present system.

Let us examine the implications of the law of chemical equilibria, however, as applied to the hypothesis that histamine combines in mono-monomolecular fashion with the receptor while two molecules of the antagonist combine in stages with the receptor. Application of the law to this competitive system yields the following equation:

$$\frac{HR}{R_T} = \frac{H}{H + \alpha + \frac{\alpha}{\alpha'} B + \frac{\alpha}{\alpha' \alpha''} B^2},$$

where HR = response of the strip in mm.
R_T = maximum response of strip in mm.
H = concentration of histamine in mols/liter
B = concentration of antagonist in mols/liter
$\alpha, \alpha', \alpha''$ = dissociation constants for the three equilibria.

This equation states that the concentration-response curves for histamine in the presence of varying concentrations of the antagonist should be a family of hyperbolas, all of which pass through the origin, have the same maximum, and differ only in their curvature, the latter being a function of the concentration of the antagonist. This relationship also states that the concentration of histamine required to produce a particular response of the strip varies in parabolic fashion with the concentration of the antagonist. Such a parabolic, constant-response isobole serves to differentiate this competitive system involving several equilibria from the simple systems involving two equilibria, in which case the isoboles are linear.

The next step is to determine quantitatively how well the experimental data conform to the equation derived from the chemical hypothesis just stated. For this purpose, correlation analysis is in order and has been performed by the statistical method of multiple correlation. The excellence of the agreement of the data with the chemical hypothesis is revealed by the magnitude of the multiple correlation coefficients, some of which are as high as 0.99.

It is, thus, apparent that the mechanism of action of the histamine antagonists can be explained on the basis of a particular system of chemical equilibria. It is also apparent that it will be necessary to determine three affinities, one of histamine for the receptor, one of the first molecule of antagonist for the receptor, and one of the second molecule of antagonist for the receptor.

The partial regression coefficients derived from the statistical fitting provide the best estimate of the numerical values for the three affinities involved in the competitive system. Examples may be given:

$$H + R \leftrightharpoons HR \quad \frac{1}{\alpha} = 0.40 \times 10^6,$$

$$B + R \leftrightharpoons BR \quad \frac{1}{\alpha'} = 7.70 \times 10^6,$$

$$\text{and } B + BR \leftrightharpoons B_2R \quad \frac{1}{\alpha''} = 0.35 \times 10^6.$$

These values are for Benadryl, and, in a limited series, those for Pyribenzamine are of the same order of magnitude.

It may be inferred from these figures that the affinity of the first molecule of antagonist for the receptor is less than that of histamine. These affinities, while they are a function of the type of receptor, are independent of the actual responses and particular concentrations of histamine or antagonist used in their determination. Thus, their proper application should provide correct estimates of potency.

The potency of histamine, in terms of its affinity for the receptor, may be expressed as the concentration of histamine required to half-saturate the receptor in the absence of the antagonist, a value which, in the present scheme, is numerically equal to α. The "potency" of the antagonist, in terms of its affinity for the receptor, may also be expressed as the concentration of antagonist necessary for half-saturation of the receptor in the absence of histamine. Since two affinities exist in the case of the antagonist, the numerical value for the half-saturation concentration in the present system is equal to $\dfrac{-\alpha'' + \sqrt{(\alpha'')^2 + 4\alpha'\alpha''}}{2}$.

In competitive inhibition, however, the effectiveness of an antagonist is not solely a function of its affinity for the receptor, but also involves the affinity of the active drug for the receptor. Thus, the proper measure of the effectiveness of an antagonist should be based on the relative affinities of the competing compounds. The relative affinity is most logically expressed as the ratio of the half-saturation concentration of histamine to the half-saturation of the antagonist. It is proposed that this ratio, which characterizes the competitive system, rather than the antagonist alone, be designated the index of competition.

In general form this competition index is

$$\text{C. I.} = \frac{[\text{H}]_{50\%}}{[\text{B}]_{50\%}},$$

and for the particular equilibrium situation under consideration, the competition index takes the form:

$$\text{C. I.} = \frac{2\alpha}{-\alpha'' + \sqrt{(\alpha'')^2 + 4\alpha'\alpha''}}.$$

Employing the previously designated values for the various dissociation constants, the competition index for Benadryl is 20.1, which implies that, in this competitive system, the affinity of Benadryl for the receptor is 20.1 times that of histamine. It should not be forgotten that this index may be different on another tissue.

GENERAL DISCUSSION. II

By Bret Ratner

New York Medical College, New York, N.Y.

I want to thank the Chairman for asking me to join in the discussion of this monograph. My first impulse would be to voice my agreement with many of the viewpoints expressed here, and to terminate my remarks at that point.

This author has, for many years, held to the view that real progress in clinical allergy would be made largely in the domain of animal experimentation. For my own part, I have probably learned more about human allergy from our studies in the guinea pig on experimental asthma, sensitization *in utero*, and passage of proteins through various living membranes than could have been arrived at from studies in the clinic.

It is well, therefore, at a conference on the subject of histamine to emphasize the importance of the immunologic basis of the hypersensitive phenomenon and bring again to our attention the three fundamental criteria of allergy: (1) the dependency upon substances known to be antigenic; (2) specificity; and (3) participation of antibodies.

Bearing these three facets in mind, namely antigenicity, specificity, and antibody participation, I might here project the problem from the lower animal to the clinic. We know that allergy is an ever present battle against the invasion into the body of foreign substances inhaled, ingested, or injected. The amelioration of allergy is based on the ability of the body to produce enough antibodies so that they may eventually reach the circulation and act as blocking or neutralizing bodies. To this end the body must continually manufacture immunizing agents. There is no single drug or substance which I can envisage as being capable of dispelling this specific antigen-antibody mechanism which is so inherent a part of the animal economy.

The hereditary atopic theory, which sought to separate human allergy from anaphylaxis in the lower animal, has been rudely brought to a halt by the observations that the atopic reagins (Prausnitz-Kustner antibodies) are found not only in the spontaneous allergies of asthma, hay fever, and urticaria occurring in man, but have also been found in the artificially induced serum sickness, *Ascaris* sensitivity, insulin sensitivity, and certain drug sensitivities, some physical allergies, as well as in hypersensitive states in the lower animal.

Animal work emphasizes anew that it is the study of the many ways and means that antigens have of invading the body and producing interreactions with tissue antibodies which offer the greatest hope for good and lasting results.

Such studies entail a search for all offending substances, their elimination or reduction, and a definite program for building up tolerance. The control of environmental excesses, highly antigenic food excesses, promiscuous and thoughtless use of sera and drugs, and control of diseases which tend to increase the permeability and dysfunction of our protecting membranes are all measures which tend to reduce the incidence of allergy.

All the papers in this monograph have emphasized the fact that the antihistaminic drugs can do no more than allay certain minor phases of allergic symptomatology. Were we to abandon the antigen-antibody hypothesis in favor of the histamine concept as the primary *modus operandi* of the allergic reaction, there would be little hope for the control and prevention of allergy.

Clinical trial of the antihistaminic drugs has now shown what they are capable of doing. They have, indeed, proved to be an enviable group of drugs that work splendidly in the alleviation of symptoms of pruritus, nasal and lachrymal secretions, and edema, but they have failed to relieve asthma or the more serious forms of dermal allergy. It is these latter manifestations, however, that comprise the largest number of allergic cases. From their mode of action, it is obvious that these antihistaminic drugs can act only as palliative agents and do not eliminate the basic mechanism responsible for allergic symptomatology.

From now on, greater emphasis should be laid on prosecuting studies in the lower animal with these antihistaminic drugs. Several groups of investigators have published some work which showed that, although antihistaminics almost uniformly prevented the intoxicating symptoms of histamine shock from occurring in the rabbit under experimental conditions, they failed to prevent specific anaphylactic shock symptoms from occurring in rabbits and guinea pigs sensitized and shocked by egg white. These authors pose the following problem: If the principal symptoms of shock and the cause of death attendant on anaphylactic shock are ascribable to the release of histamine, the amelioration of anaphylactic shock by antihistaminics should parallel their effects on histamine shock. The findings of these authors indicate that such was not the case. Whether this work will be corroborated or refuted in the future will be watched with interest.

In the light of such experiments, I should like to close my remarks by re-emphasizing the fact that the allergic manifestations of the lower animal exhibit complexities approaching, in a measure, those of the human subject, and analysis of them may uncover a wider biological meaning.

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